THE MECHANISM OF THE PINACOL–PINACONE REARRANGEMENT*

IV. EXCHANGE AND REARRANGEMENT WITH HYDROGEN ISOTOPES

By J. F. DUNCAN† and K. R. LYNN‡

Summary

The two hydroxyl atoms of pinacol exchange very rapidly and quantitatively with ^3H_2O even at room temperature. The rate of the pinacol-pinacone rearrangement in 99·7 per cent. D_2O with unibasic acids at 100 °C is about 1·9 times faster than that obtained in ordinary water.

Although these results conform with the generally accepted mechanism, some features are unexpected.

I. INTRODUCTION

The conversion of pinacol to pinacone in the presence of aqueous acids is first order with respect to the hydrogen ion concentration (Duncan and Lynn 1956) and it is generally assumed (Wallis 1947; Ingold 1953) that the first step in the reaction is the formation of the pinacol conjugate acid. If this step is rapid and not rate determining, we should expect a rapid exchange reaction between the hydroxylic hydrogen atoms and acids. And the rate of rearrangement in deuterated water should not be greatly different from that in ordinary water. The present paper describes work which confirms these expectations.

Koizumi and Titani (1938) have shown that rapid exchange of the hydroxyl hydrogen atoms of ethylene glycol with deuterated water occurs in acid, alkaline, or neutral media at 100 °C. Likewise Hine and Thomas (1953) and independently Kwart, Kuhn, and Bannister (1954) report immeasurably rapid exchange of monohydric alcohols in neutral deuterated water at 25 °C (but cf. Orr 1936). One might expect therefore that, if exchange of pinacol with water does occur, it will be rapid even at room temperature. Experiments with ^3H_2O were made to check this. The rate of rearrangement in deuterium oxide was measured at 100 °C, where the kinetics of the reaction have been studied in detail by Duncan and Lynn (1956).

II. EXPERIMENTAL

(a) Exchange Reaction

Anhydrous pinacol (∼100 mg, prepared as previously described by Duncan and Lynn (1956)) was mixed with a known weight of tritiated water at room temperature. The tritiated water was obtained by dilution with distilled

A

water of material supplied by A.E.R.E., Harwell, until the activity was
7·1 mc/ml. The pinacol was used in two concentrations : (i) with sufficient
tritiated water to form pinacol hydrate ($C_6H_{14}O_2.6H_2O$) and (ii) with sufficient
tritiated water to obtain complete solution. Within 2 min of mixing, the
reaction tube was attached to the vacuum line, which was evacuated with the
reaction tube cooled to −183 °C. Water was then removed by pumping to
less than 10^{-3} mm after removal of coolant. Samples of type (i) were completely
dehydrated in about 1 hr, those of type (ii) required about 3 hr before anhydrous
pinacol was obtained. The purity of the resultant material was checked by

TABLE 1

EXCHANGE OF ^{3}H BETWEEN PINACOL AND WATER

σ = Standard deviation

Wt. Pinacol (mg) in Mixture	Wt. ^3H_2O (mg) in Mixture	Wt. Pinacol (mg) for Assay	Observed Activity (counts/sec ± σ)	Calculated Activity (counts/sec ± σ)
	124·2*	106·0	660 ± 15	Standard
121·2	100·6	94·0	110 ± 3·1	110 ± 3
116·3	108·7	101·5	114 ± 2·9	126 ± 5
125·7	103·2	92·6	100 ± 3·0	108 ± 4
	151·3*	109·8	6810 ± 60	Standard
	99·6*	104·6	4940 ± 30	Standard
127·4	118·5	105·1	1127 ± 30	1144 ± 47
116·8	106·5	97·2	982 ± 20	1051 ± 51
123·9	119·3	103·5	1192 ± 14	1138 ± 100
	40·4	95·4	2820 ± 50	Standard
107·1	89·2	102·0	1240 ± 30	1320 ± 70
133·6	99·26	85·4	1220 ± 25	1290 ± 50
130·8	1030·5	61·8	1120 ± 30†	1270 ± 40
111·1	1072·8	90·2	2900 ± 35†	3040 ± 50

* Assay.

† In these experiments excess ^3H_2O was used. In the remainder, only enough ^3H_2O to form pinacol hydrate was used.

melting point determination. A known weight of " freeze-dried " pinacol
(60–100 mg) was dissolved in 10 ml purified dioxan (Vogel 1948) containing
0·6 per cent. p-terphenyl which had been recrystallized from ethanol to m.p.
213 °C. After standing the assay sample in flat-based stoppered containers in
the dark for 24 hr, the tritium activity was assayed by liquid scintillation
counting. Owing to the low energy of β-particles from ^{3}H, the output pulse
amplitudes were not much larger than electronic noise. The efficiency of
counting was therefore low (<2 per cent.). In addition, long-term drifts in
component characteristics caused the reproducibility to be no better than

± 10 per cent. As a check on instrumental performance, a solution of tritiated water in dioxan was used as a standard.

Preliminary results showed that a tenfold increase in pinacol concentration of the assay sample only lowered the counting rate by about 5 per cent. of its original value. In all assays, the variation in pinacol concentration was less than threefold.

The extent of exchange was measured by comparing the ^{3}H activity of samples with that of standard solutions of pinacol and tritiated water, dissolved in the dioxan–p-terphenyl solution for measurement. It had previously been shown that the presence of water did not affect the scintillating properties of the solution significantly provided the volume present was less than 3 per cent. of the dioxan volume. It was therefore assumed that the activity obtained with the standard mixtures was the same as that obtained with pinacol of the same total tritium content in the absence of water.

The results with mixtures of pinacol and tritiated water are quoted in Table 1. The values under " calculated " are obtained from the standard by assuming that complete exchange occurs between the hydrogens of the hydroxyl groups of pinacol and those of water.

(b) Rearrangement in Deuterium Oxide

The method was identical with that described earlier (Duncan and Lynn 1956) except that 99·7 per cent. " Norsk Hydro " deuterium oxide was used

TABLE 2

FIRST-ORDER RATE CONSTANTS IN DEUTERIUM OXIDE AT 100 °C

Catalyst (moles/l.)			D_2O (%)	$(k_{exp})_{D_2O}$ (sec$^{-1} \times 10^5$)	$(k_{exp})_{H_2O}$ (sec$^{-1} \times 10^5$)	$\dfrac{(k_{exp})_{D_2O}}{(k_{exp})_{H_2O}}$
Hydrochloric acid						
0·126	..	..	99·7	18·9	10·1	1·87
0·270	..	..	95	41·8	22·0	1·90
0·210	..	..	85	33·0	16·8	1·96
0·270	..	..	75	43·8	22·0	1·98
Sulphuric acid						
0·85	..	..	95	256	80	3·20
0·80	..	..	75	128	71	1·82

as the solvent. The pinacol was of normal isotopic abundance. To minimize dilution of deuterium, the acid solutions were made up by adding concentrated acid to heavy water.

The acids were purified and standardized as described earlier (Duncan and Lynn 1956). The reaction was followed by determining the residual pinacol by periodate oxidation and titrimetry (Duncan and Lynn 1956). The mean first-order rate constants for a series of such measurements are compared in Table 2 with those expected in water under the same conditions (Duncan and Lynn 1956).

III. Discussion

(a) Tritium Exchange

Table 1 shows that, within the duration of the experiment, complete exchange occurs between water and both hydroxyl groups of pinacol. As expected (Brodskii 1949), no exchange occurs between water and the methyl hydrogens. On these results, one cannot, of course, eliminate the possibility of exchange occurring in the 1 hr period required for freeze-drying. But we believe that the results found are due to almost complete exchange at room temperature in 2 min. This detail is of no significance to our present argument, however. For, whenever the exchange occurs, it is still much faster than the rate of pinacol-pinacone rearrangement in all dilute acid solutions studied (Duncan and Lynn 1956) below 100 °C. This result is therefore in accordance with the initial formation of the pinacol conjugate acid by an acid-base pre-equilibrium with the pinacol.

(b) Reactions in Deuterium Oxide

Table 2 shows that there is a large increase in the rate of reaction of pinacol in deuterium oxide. This confirms the results of Ley and Vernon (1956), who obtained a 2·2-fold increase in rate with 1·86M sulphuric acid at 72·9 °C with 1,2-dihydroxy-2-methylpropane. A difference in rates might have been expected from one of two causes.

(i) *Simple Isotope Effect.*—If the hydrogen atom which is replaced by deuterium takes part in the rate-determining step of the rearrangement, we should expect a decrease in the reaction rate of several-fold. The results of the tritium exchange reaction require that the deuterium replaces hydrogen in both hydroxyl groups. Thus neither of these atoms is concerned with the rate-determining step. The results imply not only that the initial pre-equilibrium

$$P + H_3O^+ \rightleftharpoons PH^+ + H_2O \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

(where P refers to the pinacol molecule) is rapid, but also that the final stage of the reaction, in which the second hydrogen atom is lost, is not involved in the rate-determining step.

(ii) *Differences of Acidity Constant.*—If we assume that the equilibrium (1) is the first step in the reaction, then the rate of rearrangement can be shown to be (Duncan and Lynn 1956)

$$d[P]/dt = -k[HP^+] = -k.K_a[H^+][P]\gamma_{H^+}\gamma_P/\gamma_{HP^+}, \quad \ldots \quad (2)$$

where $K_a = 1/K_H$, K_H being the acidity constant of the pinacol conjugate acid, and the remaining symbols are as described earlier (Duncan and Lynn 1956). The activity coefficient terms in D_2O and in ordinary water would not be expected to differ by more than a few per cent. All differences in reaction rate with strong unibasic acids must therefore be ascribed to changes in K_H as the percentage D_2O is altered. It would therefore appear that, if the pre-equilibrium mechanism is accepted, then $K_H/K_D \sim 1·9$.

There are, however, two facts which suggest that such a simple interpretation may not be correct. Firstly, $(k_{exp})_{D_2O}/(k_{exp})_{H_2O}$ falls as the percentage D_2O in the solvent increases from 75 to 99·7 per cent. A falling value of $(k_{exp})_{D_2O}/(k_{exp})_{H_2O}$ can be obtained (Bell 1941) in this region with $(k_{exp})_{D_2O}/(k_{exp})_{H_2O} \sim 1 \cdot 1$, but one would have expected such high values of $(k_{exp})_{D_2O}/(k_{exp})_{H_2O}$ to increase steeply with increasing percentage D_2O. Secondly, from the work of Martin and Butler (1939), a value of $K_H/K_D = 1 \cdot 9$ would require K_H to have a value of about 1, that is, the pinacol conjugate acid would be a much weaker acid than H_3O^+ ($K_H = 55 \cdot 5$). Recent work (Gurney 1953 ; Wicke, Eigen, and Ackermann 1954 ; Conway, Bockris, and Linton 1956) has shown that the acidity constants of methanol, ethanol, and n-propanol are respectively 242, 945, and 1500. If the pinacol conjugate acid were similarly a strong acid compared with H_3O^+, we should expect K_H/K_D to be much smaller than observed. A more careful examination of these features is thus warranted.

The results with sulphuric acid are not germane to this argument. For, if we take the acidity constant (Duncan and Lynn 1956) of the HSO_4^- ion to be 10^{-2}, interpolation from the results of Martin and Butler (1939) leads to a value for the acidity constant of the DSO_4^- ion of 4×10^{-3}. Therefore, in $0 \cdot 8M$ sulphuric acid in D_2O, the $[SO_4^{2-}]$ would be $0 \cdot 003M$ compared with $0 \cdot 008M$ in H_2O. Whilst these concentration changes may be just acceptable in lower sulphuric acid concentrations (Duncan and Lynn 1956), a very large decrease in the rate of the reaction from activity effects alone (Duncan and Lynn 1956) can be obtained with sulphate ion concentrations of the order of $0 \cdot 02M$. Interpretation of kinetic measurements in sulphuric acid is therefore difficult. In particular, the increase in rate observed in D_2O will be due in part to the decrease in SO_4^{2-} concentration. Since the magnitude of this effect is unknown, the argument on the mechanism has been confined to strong unibasic acids.

(c) Significance for the Reaction Mechanism

If one accepts that the reaction proceeds through the acid-base pre-equilibrium, this work provides supporting evidence that the first and last stages at 100 °C are
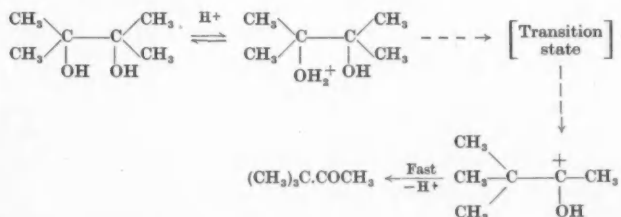
This mechanism is in accordance with the following : (i) the experimental rate equation (Duncan and Lynn 1956), (ii) the rapid exchange rate of pinacol with tritium, (iii) the absence of decrease in reaction rate in D_2O compared with H_2O. The magnitude of the reaction rate in D_2O and its variation with D_2O concentration seem however to be unexpected.

IV. Acknowledgments

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THE MECHANISM OF THE PINACOL–PINACONE REARRANGEMENT

V. CARBON ISOTOPE EFFECTS

By J. F. Duncan* and K. R. Lynn†

Summary

The intermolecular isotope effects of methyl-labelled and alcoholic carbon-labelled pinacol converted to pinacone have been studied over a temperature range of 60–113·5 °C. Tests were made to establish the validity of the results by using two starting materials for synthesizing methyl-labelled pinacol, and several different methods of analysing the products. The isotope effects were determined by measurements made both on (i) pinacol and (ii) pinacone.

The results above 80 °C can be interpreted in terms of the synartetic ion mechanism provided both the carbonium ion and the transition state are largely unhydrated, and both the formation of the carbonium ion and the methyl migration are slow steps.

Below 60 °C an alternative mechanism is operative. Unless unsuspected exchange reactions are present, the results suggest that the transition state is not so greatly distorted from a simple ethane-type configuration as in the high temperature reaction. Results at intermediate temperatures indicate that the alternative route proceeds via a stable intermediate.

I. Introduction

In previous work we have shown that at 100 °C the mechanism of the conversion of pinacol to pinacone may be written
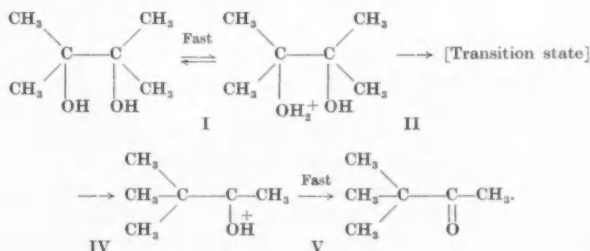
Having established the validity of steps I and V, we next discuss step II, which is commonly believed to involve the carbonium ion
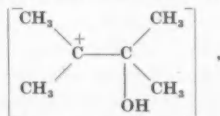
* Chemistry Department, University of Melbourne.

† Tracer Elements Investigations, C.S.I.R.O.; present address: Chemistry Department, University of Wisconsin.

and the synartetic ion

$$\left[\begin{array}{c} CH_3 \\ CH_3 \overset{\cdots}{\underset{CH_3}{\overset{|}{C}}} \overset{\cdots +\cdots}{\text{———}} \overset{CH_3}{\underset{OH}{\overset{|}{C}}} CH_3 \end{array} \right].$$

Evidence related to this stage of the reaction has been obtained by two types of investigation. Firstly, the exchange react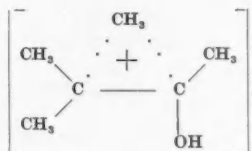ion of pinacol with $H_2^{18}O$ has been investigated and shown to be faster than the rate of rearrangement (Duncan and Lynn, unpublished data ; see also Bunton *et al.* 1956). Secondly, carbon-labelled isotope effects have been determined and are discussed in the present paper. Although primarily directed at elucidation of the mechanism at 100 °C, this work has also led to useful conclusions about the reaction at 60–80 °C.

The relative reaction rates of isotopic molecules may be determined by measuring the changes in isotopic composition of either the product or the reactant at various stages of the reaction. We have used both these methods in this reaction, with ^{14}C-labelling of either the methyl group, or the alcoholic carbon atom.

In this paper the carbon atoms of the pinacol molecule are referred to by the following numbers :

$$_1CH_3 \text{———} \overset{OH}{\underset{_5CH_3}{\overset{|}{_2C}}} \text{———} \overset{OH}{\underset{_6CH_3}{\overset{|}{_3C}}} \text{———}_4CH_3$$
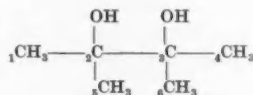
The $_2C$ and $_3C$ are also called " alcoholic carbon atoms " for convenience. The remainder are called " methyl carbon atoms ".

II. EXPERIMENTAL

(a) *Measurement of Residual Reactant*

Since there is no convenient precipitant for pinacol, it was assayed by quantitative oxidation with periodate to yield acetone, which could then be precipitated as its 2,4-dinitrophenylhydrazone. An isotope effect of 10 per cent. has been reported by Brown and Holland (1952) for the hydrazone precipitation of carbonyl-^{14}C benzophenone. Using their method, we have observed a 7 per cent. isotope effect with acetone ; but this is eliminated by use of dinitrophenyl-hydrazone hydrochloride in excess, sufficient to give greater than 99 per cent. precipitation. No isotope effect is therefore introduced in our experiments with this technique. Reproducibility of precipitation was shown to be within the limits of statistical variation of the counting rate, by precipitation of identical samples of carbonyl-^{14}C acetone.

Aliquots of pinacol were made up which contained $0 \cdot 7$–$1 \cdot 5$ mg of the original synthesized labelled pinacol in 8–17 mg (made up by dilution with

pinacol of normal isotopic abundance). The aliquots were diluted with acid of known strength to 25 or 50 ml. Either 2 or 5 ml samples of each dilution were sealed in tubes, which were immersed in a thermostat (Duncan and Lynn 1956) at zero time. They were removed at known fractions of complete reaction (Duncan and Lynn 1956) and cooled in ice-water.

Several methods of preparing the reaction mixture and analysing the products of reaction were used.

(i) An aliquot of radioactive pinacol in acid was diluted with inactive pinacol to form a $0 \cdot 1M$ solution. This solution was divided into several samples, each of which was used to determine the degree of reaction of the labelled pinacol at a different time. To analyse the products, excess periodate-bicarbonate mixture was added to each sample. The solution was allowed to stand for 4 hr ; 1 ml of $0 \cdot 85M$ acetone in water was added, and excess periodate and iodate precipitated with saturated barium hydroxide. After centrifuging, the supernatant liquid was decanted (with washings) into excess of a saturated solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid. After standing for an hour in an ice-bath, the hydrazone was filtered, washed, and dried under vacuum over concentrated sulphuric acid. The dry acetone-2,4-dinitrophenylhydrazone samples were recrystallized several times, with filtering, from ethanol-water mixtures until the measured activity of each sample was constant within statistical variation. Chromatography on Whatman No. 2 paper with petroleum-methanol mixtures (Meigh 1952) showed that no pinacone dinitrophenyl-hydrazone remained.

(ii) An aliquot of labelled pinacol in aqueous acid solution was allowed to react without addition of inactive pinacol. After the reaction, a $0 \cdot 1M$ solution of inactive pinacol was added in amount proportional to the percentage residual reactant, choosing 60 mg as 100 per cent. The subsequent treatment was identical with method (i).

(iii) This method was similar to method (ii), except that $0 \cdot 1M$ solution of acetone was used for the dilutions, in amounts proportional to the weight of residual reactant. In the oxidation of pinacol, only sufficient periodate was used to complete the oxidation, and no precipitation of excess periodate was made. The acetone-2,4-dinitrophenylhydrazones were recrystallized twice with methanol or ethanol. Aliquots of each batch of crystals were diluted with measured amounts of pure inactive 2,4-dinitrophenylhydrazone and the mixtures purified usually by recrystallization, using the same solvents, or light petroleum, but in some randomly selected cases by chromatography. The chromatographic method was also used on one occasion after dilution with inactive pinacol as described in (ii). In no case were any contaminants found.

(iv) Samples were prepared, reacted, and diluted by method (ii). The resulting mixtures were made alkaline with bicarbonate, and water and other volatiles distilled off at 0 °C on a vacuum line. The residual pinacol was oxidized with periodate, and treated as in method (i). Purity was again checked by isotope dilution and chromatography.

(v) In one experiment, the change in specific activity of the reacting pinacol was followed for a time about twice as long as required for 95 per cent. complete

reaction. In each extraction, about 60 mg pinacol carrier was added, the radioactive pinacol oxidized to acetone, and the specific activity of the 2,4-dinitrophenylhydrazone determined. The results of this experiment are quoted in Table 5.

(b) Measurement of Cumulative Product

The reactant mixtures were prepared as in method (ii) and sampled as in method (i). The products were diluted with purified pinacone in water, in proportion to the reactant formed (100 per cent. = 50 mg). After filtration, washing, and vacuum drying over concentrated sulphuric acid, the precipitate was recrystallized from methanol, ethanol, or a mixture of light petroleum and benzene until the measured activity was constant within statistical variation.

Auxiliary experiments showed that the pinacone 2,4-dinitrophenylhydrazone could be precipitated by the procedure of Iddles and Jackson (1934) in 93–95 per cent. yield, with reproducibility within the statistical variation of activity measurement. Use of excess 2,4-dinitrophenylhydrazine eliminated the possibility of isotope effects in the precipitation. It was shown that no significant further rearrangement occurred during the formation of the 2,4-dinitrophenylhydrazone (1 hr at room temperature) in spite of the presence of pinacol and acid together.

(c) Radioactive Assay

After filtration, the samples (~60 mg total mass) were packed manually into 2 cm^2 planchets of polythene. All samples were of effectively infinite thickness. Reproducibility in counting rate with standard samples was about 3–5 per cent. Each sample was assayed for a total of 3000–5000 counts with a mica-windowed bell-jar type Geiger Müller counter.

(d) Materials

Except where explicitly stated below, all reagents were prepared and purified as described earlier (Duncan and Lynn 1956). Pinacone used as diluent was prepared from pinacol (Organic Synthesis 1943) and purified by distillation (b.p. 106 °C). The 2,4-dinitrophenylhydrazine hydrochloride solution (~5 g/l) was prepared from A.R. grade reagents using 2N hydrochloric acid. The solvents for chromatography and crystallization were redistilled in all-glass apparatus before use. Saturated barium hydroxide solutions were made from L.R. material, filtered, and stored under soda lime.

(i) ^{14}C-Methyl-labelled Pinacol.—About 32 mg $^{14}CH_3I$ (1·05 mc) obtained from the Radiochemical Centre, Amersham, was distilled onto 1·47 g A.R. methyl iodide in a vacuum manifold. After mixing, the combined methyl iodide was distilled into 0·5 g elemental magnesium in 30 ml dry ether under an ice-cooled condenser. Dry CO_2-free air was introduced into the reaction flask, which was removed from the vacuum line, and 0·6 g diacetyl (b.p. 88 °C) in 30 ml dry ether was added with rapid stirring. After standing for 12 hr, the reaction mixture was decomposed with dilute sulphuric acid. Sodium thiosulphate was added, to remove free iodine, and the labelled pinacol was extracted continuously with purified ether.

The ether extract was dried over calcium sulphate and distilled, yielding 0·41 g pale yellow oil, which produced white crystals on addition of water. This product was recrystallized from water to yield 0·69 g of white tablets, m.p. 42 °C (pinacol hexahydrate).   Radioactive yield (610 μc)=58 per cent.

Purity was checked by paper chromatography, using an *n*-butanol-water mixture (Hough 1950). Scanning the paper chromatogram under a Geiger counter showed the presence of one radioactive compound only, and this was in a similar position to that obtained in a separate experiment with inactive pinacol. A few milligrams of the active material were mixed with inactive pinacol hexahydrate, and the product recrystallized from water.   No drop in specific activity was observed on repeated recrystallization.

Methyl-labelled pinacol was also synthesized from 0·8 mc $^{14}CH_3OH$.   It was converted to the bromide by Foreman, Murray, and Ronzio's (1950) method and the product purified and dried as they recommended.   The pinacol was then synthesized, purified, and tested for impurities as described above.   Yield 545 mg, containing 560 μc (70 per cent. radioactive yield).

(ii) *Alcoholic Carbon* ^{14}C-*Pinacol.*—^{14}C-Carbonyl acetone (697 mg ; from Radiochemical Centre, Amersham) was distilled in a vacuum manifold into a centrifuge tube (containing 0·3 g magnesium, 0·3 g A.R. mercuric chloride, and 2·3 ml purified benzene) connected by a cone-socket joint.   Air (dried over calcium chloride-soda lime mixture) was introduced into the manifold.   The tube was rapidly disconnected, and re-connected by the cone-socket joint to a condenser and dropping funnel, protected with tubes of pelleted calcium chloride. Slight warming caused the reaction to begin.   Heating was continued for 2 hr, and the reaction mixture was then decomposed with 2 ml distilled water.   The benzene solution was separated.   The aqueous layer was extracted twice more with hot benzene, and all pooled extracts were concentrated to about 2 ml. Water was added and the resultant white crystals were collected, purified, and tested for impurities as described above.   Yield 326 mg (24 per cent.) containing 23·2 μc.

III. RESULTS

To determine the value of $\varepsilon_{exp}=k^*/k$, where k is the reaction rate of the isotopically normal molecule, and k^* refers to the labelled-molecule, one of two methods is possible :

(i) The specific activity of the pinacol at zero time (S_0) may be compared with the specific activity of the pinacol when a fraction γ of the reaction has been completed ($(S_\gamma)_R$.   Since

$$(S_\gamma)_R/S_0 = (1-\gamma)^{[\varepsilon_{exp}-1]}$$

(Bigeleisen 1949 ; Downes and Harris 1952), the isotope effect ε_{exp} may be obtained by plotting log $[(S_\gamma)_R/S_0]$ against log $(1-\gamma)$.   The slope of the resulting straight line is equal to $\varepsilon_{exp}-1$.

(ii) The specific activity of the resulting pinacone at effectively complete reaction (S_∞) may be compared with the resulting pinacone when a fraction γ

of the reaction has been completed $(S_\gamma)_P$. Since (Bigeleisen 1949 ; Downes and Harris 1952)

$$(S_\gamma)_P/S_\infty = [1-(1-\gamma)^{\varepsilon_{exp}}]/\gamma,$$

a plot of $\log [1-\gamma(S_\gamma)_P/S_\infty]$ against $\log (1-\gamma)$ again gives a straight line of slope equal to ε_{exp}.

Tables 1–10 present the results obtained. In all these tables the letters A, B, C, etc. refer to a different series of experiments. The value of γ was taken from kinetic results reported earlier for temperatures above $67 \cdot 8$ °C (Duncan and Lynn 1956). Kinetics at lower temperatures have been investigated and will be reported separately.

TABLE 1

14C-METHYL PINACOL AT $113 \cdot 5$ °C WITH $0 \cdot 2$N HYDROCHLORIC ACID

γ	From Pinacol $\left[\log \dfrac{(S_\gamma)_R}{S_0}\right]$			From Pinacone $\left\{-\log\left[\dfrac{S_\infty - \gamma(S_\gamma)_P}{S_\infty}\right]\right\}$		
	A	B	C	A	B	C
0·1	0·031					
0·2	0·058		0·071	0·017	0·012	0·011
					0·018	0·001
0·3	0·095					
0·4	0·141	0·140	0·138	0·064		0·066
		0·128	0·144			0·064
0·5	0·169		0·209			0·124
			0·211			0·106
0·6	0·211			0·173	0·164	
0·7	0·267	0·230	0·264	0·250	0·236	0·187
						0·181
0·8	0·321	0·316	0·341	0·349	0·382	0·28
		0·311	0·357			0·352
0·9				0·458		

TABLE 2

ALCOHOLIC 14C-PINACOL AT $113 \cdot 5$ °C WITH $0 \cdot 2$N HYDROCHLORIC ACID

γ	From Pinacol $\left[\log \dfrac{(S_\gamma)_R}{S_0}\right]$		From Pinacone $\left\{-\log\left[\dfrac{S_\infty - \gamma(S_\gamma)_P}{S_\infty}\right]\right\}$	
	A	B	A	B
0·1	0·01	0·013		
0·2	0·024	0·024	0·015	0·016; 0·015
0·3	0·040	0·031		
0·4	0·059	0·049	0·061	
0·5		0·073		
0·6	0·13	0·12	0·195	0·191; 0·195
0·7	0·15	0·15	0·246	0·308; 0·311
0·8	0·20	0·21	0·501	0·408; 0·429
0·9				0·722; 0·571

TABLE 3

14C-METHYL PINACOL AT 100 °C WITH 0·2N HYDROCHLORIC ACID

TABLE 3

^{14}C-METHYL PINACOL AT 100 °C WITH 0·2N HYDROCHLORIC ACID

The primed letters refer to experiments made with pinacol synthesized from $^{14}CH_3OH$; the unprimed from $^{14}CH_3I$

| γ | From Pinacol $\left[\log \dfrac{(S_\gamma)_R}{S_0}\right]$ | | | | | | From Pinacone $\left\{-\log\left[\dfrac{S_\infty-\gamma(S_\gamma)_P}{S_\infty}\right]\right\}$ | | | |
| | 0·2N HCl | | | | | 0·01N HCl | 0·2N HCl | | | 0·01N HCl |
	A	B	C	D'	E'	A'	A	B	C'	A'
0·10	0·035	0·041		0·059		0·045, 0·028				
0·15		0·068								
0·20		0·093	0·053	0·097	0·068	0·060, 0·043	0·02	0·022, 0·014	0·03, 0·017	0·04
0·25	0·117	0·093			0·113, 0·091					
0·30		0·104		0·068	0·083		0·047	0·053	0·046, 0·040	
0·40	0·146	0·137			0·106, 0·128	0·120				0·116
0·45					0·153					0·121
0·50	0·188		0·182	0·175	0·163, 0·173	0·156	0·123	0·159	0·121, 0·118	0·136
0·60		0·253		0·222	0·253, 0·250		0·179		0·15, 0·153, 0·190	
0·70	0·270		0·288	0·284	0·274, 0·265	0·249	0·267	0·288	0·233, 0·229	0·28, 0·302
0·80			0·342	0·421			0·324	0·384		
0·87				0·410						0·50, 0·49

TABLE 4

ALCOHOLIC ^{14}C-PINACOL AT $100\,°$C WITH $0\cdot2$N HYDROCHLORIC ACID

γ	From Pinacol $\left[\log \dfrac{(S_\gamma)_R}{S_0}\right]$				From Pinacone $\left\{-\log\left[\dfrac{S_\infty-\gamma(S_\gamma)_P}{S_\infty}\right]\right\}$
	A	B	C	D	A
0·10				0·011	
0·15				0·015	
0·20	0·0231	0·0219	0·010	0·036 ; 0·039	0·02
0·30				0·045	0·042 ; 0·033
0·50	0·0851	0·0880	0·082	0·084	0·097 ; 0·098
0·60		0·0916		0·081	0·193
				0·105 ; 0·107	
0·70			0·134		0·310 ; 0·289
			0·125		
0·80		0·176	0·194		0·452 ; 0·432
			0·177		

TABLE 5

CHANGE IN ACTIVITY (a) OF PINACOL AND PINACONE IN THE REACTION OF ^{14}C-METHYL PINACOL IN $0\cdot2$N HYDROCHLORIC ACID

" a " is the measured activity at infinite thickness of the radioactive material in the same weight of carrier. The ratio of the activity in the reactants $(a_\gamma)_R$ [or products $(a_\gamma)_P$] at a fraction γ of reaction to that present at zero time $(a_0)_R$ [or at infinite time $(a_\infty)_P$] is therefore a measure of the fraction of the total activity present in the sample at the time specified

Temperature 100 °C*				Temperature 78 °C		
Time (sec)	γ	Pinacol $\left[\dfrac{(a_\gamma)_R}{(a_0)_R}\right]$	Pinacone $\left[\dfrac{(a_\gamma)_P}{(a_\infty)_P}\right]$	Time (min)	γ	Pinacone $\left[\dfrac{(a_\gamma)_P}{(a_\infty)_P}\right]$
810	0·24	0·94		240	0·10	0·017
1140	0·28		0·09	1065	0·33	0·095
1680	0·36	0·89		2835	0·64	0·64
2340	0·41		0·22	3270	0·69	0·77
3960	0·56		0·32	4335	0·80	0·80
6720	0·69	0·61		5265	0·87	0·88
9180	0·80	0·50	0·50	6675	0·95	0·99
10,800	0·88		0·68	8145	1·00	1·02
12,480	0·94	0·37		9585	1·00	0·98
13,260	1·00		0·83	13,905	1·00	0·99
15,600	1·00	0·21		15,345	1·00	1·01
15,720	1·00		0·92			
18,180	1·00	0·10				
18,240	1·00		0·94			
20,640	1·00	0·06				
23,400	1·00	0·06				
26,820	1·00	0·03				
30,000	1·00		1·02			
90,000	1·00		0·99			

* The pinacol and pinacone results quoted here were determined in separate experiments.

TABLE 6

^{14}C-METHYL PINACOL AT 78 °C WITH 0·2N HYDROCHLORIC ACID

γ	From Pinacol $\left[\log \dfrac{(S_0)_R}{S_0}\right]$			From Pinacone $\left\{-\log\left[\dfrac{S_\infty - \gamma(S_\gamma)_P}{S_\infty}\right]\right\}$		
	A	B	C	A	B	C
0·10	0·024	0·032				
0·20	0·037	0·050				
0·27				0·122		
				0·138		
0·29			0·081			0·142
			0·071			0·160
0·30	0·056					
0·35				0·188	0·222	
				0·165	0·222	
0·40		0·099				
0·54				0·342		
				0·398		
0·55					0·342	
					0·399	
0·58			0·225			0·422
						0·388
0·60	0·174					
0·605					0·399	
					0·540	
0·645				0·439		
				0·523		
0·65			0·245			0·416
						0·497
0·70	0·248					
0·74			0·322			0·610
0·75	0·301					
0·755				0·762		
0·78			0·308			
			0·339			
0·79	0·353	0·383				
0·80	0·340					
0·87						0·879
						0·994

TABLE 7

ALCOHOLIC ^{14}C-PINACOL AT 78 °C IN 0·2N HYDROCHLORIC ACID

γ	From Pinacol $\left[\log \dfrac{(S_\gamma)_R}{S_0}\right]$		From Pinacone $\left\{-\log\left[\dfrac{S_\infty - \gamma(S_\gamma)_P}{S_\infty}\right]\right\}$		
	A	B	A	B	C
0·1	0·0076	0·011			
0·2	0·010	0·026			
	0·028				
0·3	0·032	0·044	0·162		
			0·142		
0·32					0·16
0·35				0·196	
0·40	0·041	0·065			
0·50		0·099			
0·55				0·388	
0·60	0·097	0·11	0·417		
			0·415		
0·68				0·467	
				0·467	
0·70	0·12		0·544		
	0·15				
0·72					0·638
0·75		0·19			
0·76					0·618
0·79		0·21			
0·80	0·19		0·645		
	0·19				

TABLE 8

^{14}C-METHYL PINACOL AT 78 °C IN 0·2N HYDROCHLORIC ACID

In these results both the pinacol and pinacone were worked up from the *same* reaction mixture

γ	From Pinacol	$\left\{-\log\left[\dfrac{S_\infty - \gamma(S_\gamma)_P}{S_\infty}\right]\right\}$
0·05	0·042	
0·11		0·050
0·30	0·080	
0·41		0·230
0·43	0·102	
0·48		0·279
0·61	0·195	
0·69		0·510
0·87	0·420	

TABLE 9

^{14}C-METHYL PINACOL AT 60 °C WITH 0·995N HYDROCHLORIC ACID

γ	From Pinacol $\left[\log \dfrac{(S_\gamma)_R}{S_0}\right]$		From Pinacone $\left\{-\log\left[\dfrac{S_\infty - \gamma(S_\gamma)_P}{S_\infty}\right]\right\}$	
	A	B	A	B
0·125	—0·006	0·00	0·055	0·052
0·29	0·0067		0·156	—
0·33	0·007	0·024	0·174	0·159
0·44	—0·013	—0·022	0·232	0·252
0·48	0·031		0·308	
0·55	0·018		0·366	
0·58	0·002		0·394	

TABLE 10

METHODS USED TO DETERMINE ISOTOPE EFFECT AT 100 °C

Preparative Route	Extractive Procedure	Measurements on :	Catalyst (N HCl)	Observed Isotope Effect	
				^{14}C-Methyl	Alcoholic-^{14}C
1	(a) i	Residual reactant	0·2	0·47, 0·48	
1	(a) ii	Residual reactant	0·2	0·48, 0·47	0·74, 0·77, 0·77,
2	(a) iii	Residual reactant	0·2	0·47	0·77
2	(a) iii	Residual reactant	0·01	0·48*	
1	(b)	Cumulative product	0·2	0·47, 0·48	0·74
2	(b)	Cumulative product	0·2	0·47	
2	(b)	Cumulative product	0·01	0·48	

* At 78 °C.

IV. DISCUSSION

(a) Reliability of Results

Isotope effects are notoriously difficult to determine with accuracy. For not only are the differences in reaction rates between isotopic molecules usually small, but also they are very easily confused with apparent isotope effects produced by the presence of traces of impurities. In investigating isotope effects in this reaction, therefore, we have made numerous tests to confirm that the observations could not be accounted for by the presence of labelled impurities. Direct tests for possible impurities (described in Section III) were all negative. We have also measured the isotope effect in several different ways so as to be as certain as we can that the results are reliable. The methods used, with the results obtained, are summarized in Table 10. To these may be added the results at temperatures below 70 °C, which showed no evidence of an isotope effect (see Table 11), although the same methods were used for

B

isolating the pinacol and pinacone as were used at the higher temperatures when isotope effects were obtained.

<div align="center">TABLE 11</div>

<div align="center">MEAN VALUES OF ε_{exp}</div>

Temperature (°C)	^{14}C-Methyl Pinacol		Alcoholic ^{14}C-Pinacol	
	From Pinacol	From Pinacone	From Pinacol	From Pinacone
113·5	0·49	0·47	0·72	0·73
100·0	0·47	0·47	0·76	0·74
78·0	0·48	1·0	0·73	1·0
60·0	1·0	1·0	—	—

Also, the results quoted in Table 5 show (i) that less than 3 per cent. of the ^{14}C is present as impurities which do not react (pinacol results) and (ii) that greater than 94 per cent. of the ^{14}C activity which will form pinacone does so in a time equal to twice that necessary for 0·88 per cent. of reaction. Since the total activity in the system at this stage extractable as pinacol or pinacone together is equal to that originally added (see Table 5), it follows that at least 90 per cent. of the isotope effects observed are genuine.

If the isotope effects obtained at high temperatures were due to an impurity, it would need to be :

(1) produced in the synthesis after the methanol stage ;

(2) converted to pinacol after the high temperature rearrangement, that is, during the subsequent separations, but not converted to pinacone ;

(3) converted to pinacol and pinacone (either after rearrangement or during subsequent separation) in the same proportion as these species are already present during each stage of the low temperature reaction ;

(4) inseparable from pinacol and pinacone by precipitation of 2,4-dinitrophenylhydrazone, chromatography, or fractional crystallization.

Whilst one can never rule out the presence of such an impurity absolutely, no obvious impurity (e.g. tetramethyl ethylene oxide, 2,3-dimethylbutadiene, 1-methyl-2-dimethyl*cyclo*propanol) would have these properties. We believe, therefore, that the results must be explained as true isotope effects. They may however be apparent, rather than real, due to the presence of two or more simultaneous reactions not detected earlier in our work. Table 11 gives a summary of the results. It will be seen that quite different results are obtained above 78 °C from those obtained below this temperature, which shows that more than one reaction can take place. We shall consider separately the results at 100 °C (at which temperature our previous work has been done), at low temperatures where zero isotope effects are obtained and at 78 °C, which is a special case.

(b) *Isotope Effects at 100 °C*

To interpret any isotope effect one must assume a reaction mechanism. It seems reasonable in the present case to assume the conventionally accepted mechanism (which is in accordance with the observed kinetics at 100 °C), namely,
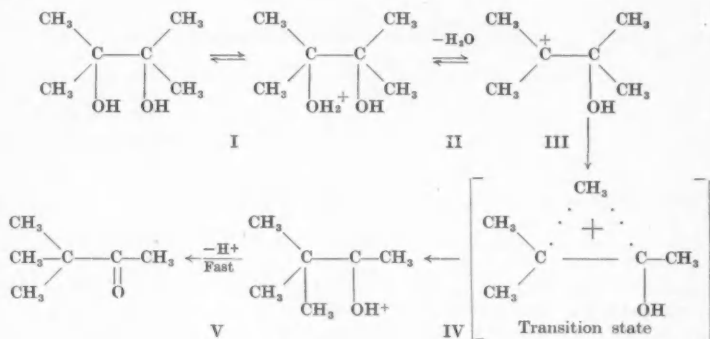
It is always possible that our assumed mechanism is incorrect, in which case our interpretation will be irrelevant. And since our work has shown some features of this reaction to be unexpected (Duncan and Lynn 1957), our interpretation of the observed isotope effects should be regarded as provisional on the acceptance of the above scheme as the sole mechanism operative at high temperatures. In this mechanism, isotope effects cannot be obtained with methyl-labelled reactant in stages I and V (since they do not involve the methyl group). And since stage V is also fast no isotope effect will be obtained in this stage even with alcoholic C-labelled pinacol. The observations must therefore be interpreted in terms of stages II, III, and IV only.

(i) *Stage II, Carbonium Ion Formation.*—Oxygen exchange experiments indicate that the C—O bond of pinacol is ruptured at a rate which is much faster than the rate of pinacone formation (Duncan and Lynn, unpublished data). This could be interpreted as indicating that the carbonium ion reaches equilibrium with the pinacol conjugate acid. It will be shown in a later paper, however, that this would lead to a change in the rate equation which is not observed. It would also disagree with the observation that the rate of conversion of pinacol to pinacone does not follow the stoichiometric hydrogen ion concentration at high acid concentrations. If such an equilibrium is not obtained, one must conclude that the activated states for the oxygen exchange and the rearrangement must be different. In the case of secondary alcohols, the oxygen exchange reaction has been shown to require only a small extension of the C—O bond, and the water molecule never leaves the sphere of influence of the carbonium ion (Bunton, Konasicwicz, and Llewellyn 1955). This accounts for the fact that (as in the case of the pinacol-pinacone reaction) water does not enter stoichiometrically into the rate equation. If the rate of formation of the

carbonium ion in a form required for the rearrangement is much slower than the rate of oxygen exchange with water, an isotope effect will be possible in stage II. Whilst the carbonium ion cannot be completely unhydrated, an isotope effect in this stage would require the binding of the water molecule to the carbonium ion to be so loose that the 2-carbon atom essentially no longer

TABLE 12

MINIMUM VALUE OF ε FOR DIFFERENT ASSUMPTIONS

4–CH$_3$ group migrates

Condition	Labelled C-atom	Stage of Reaction		
		II	III	IV
1. Hydrated carbonium ion and synartetic ion II an equilibrium..	1,5,6	1	1	1
	2	0·9	1	0·67
	3	1	0·67	1
	4	1	0·67	0·67
2. II an equilibrium : Synartetic ion unhydrated				
(a) Vibration frequencies of 1–2, 5–2, and 2–3 bonds only affected	1,5	1	0·67	0·67
	2	0·9	0·67	0·67
	3	1	0·67	1
	4	1	0·67	0·67
	6	1	1	1
(b) Vibration frequencies of whole synartetic ion affected	As 2 (a), but $(\varepsilon_4)_3 = (\varepsilon_4)_6 = (\varepsilon_3)_6 = 0·67$*			
3. (a) As 2 (a), but equilibrium not maintained in II	As 2 (a), but $(\varepsilon_2)_2 = 0·67$			
(b) As 2 (b), but equilibrium not maintained	As 2 (b), but $(\varepsilon_2)_3 = 0·67$ $(\varepsilon_2)_1 = (\varepsilon_2)_{3-6} = 1$			
4. (a) As 3 (a), but frequencies of carbonium ion and pinacol conjugate acid significantly different	As 2 (a), but $(\varepsilon_2)_n = 0·67$			
(b) As 3, but skeletal frequencies of pinacol conjugate acid, carbonium ion, and synartetic ion significantly different (minimum possible value of ε)	$(\varepsilon_m)(b) = 0·67$			
5. Concerted reaction	$2\varepsilon_2 = 2\varepsilon_3 = 2\varepsilon_4 = \varepsilon_1 = \varepsilon_5 = \varepsilon_6 = 0·67$†			

* This value must be increased by the factor $(\mu^*/\mu)^{\frac{1}{2}}$ for the 6–C atom to allow for the intramolecular isotope effect.
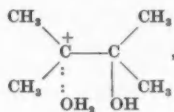
† These suffixes refer to the position of the C-atom.

has an sp^3 hybridized bond structure. In Tables 12 and 13, the values of theoretically possible isotope effects for various types of reaction are quoted sometimes with stage II as an equilibrium and sometimes when stage II involves a rate process.

(ii) *Migration Step.*—In the subsequent stages of the reaction, several possibilities exist.

(1) *Hydrated Carbonium Ion and Transition State.* If the carbonium ion is written

$$
\begin{array}{c}
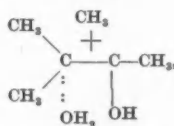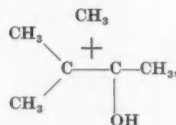CH_3 \qquad\quad CH_3 \\[2pt]
\overset{+}{C}\!\!-\!\!C \\[-2pt]
CH_3 \;\vdots\quad\; CH_3 \\[2pt]
OH_2 \; OH
\end{array}
\; ,
$$

and the transition state as

$$
\begin{array}{c}
CH_3 \quad CH_3 \\[2pt]
\overset{+}{} \\[-6pt]
C\!-\!C\!-\!CH_3, \\[-2pt]
CH_3 \;\vdots \\[2pt]
OH_2 \; OH
\end{array}
$$

TABLE 13

MINIMUM VALUES OF ε_{Total} FOR DIFFERENT ASSUMPTIONS

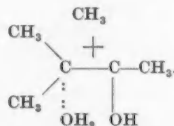| Condition	Labelled Group	ε_{Total}
1. Hydrated carbonium ion and synartetic ion II an equilibrium	Methyl Alcoholic C	0·86 0·63
2. II an equilibrium synartetic ion unhydrated (a) Vibration frequencies of 1–2, 5–2, and 2–3 bonds only affected	Methyl Alcoholic C	0·56 0·53
(b) Vibration frequencies of whole synartetic ion affected	Methyl Alcoholic C	0·44 0·42
3. (a) As 2 (a), but equilibrium not maintained in II	Methyl Alcoholic C	0·56 0·46
(b) As 2 (b), but equilibrium not maintained in II	Methyl Alcoholic C	0·44 0·37
4. (a) As 3 (a), but frequencies of carbonium ion and pinacol conjugate acid significantly different	Methyl Alcoholic C	0·39 0·37
(b) As 3 (b), but skeletal frequencies of pinacol conjugate acid, carbonium ion, and synartetic ion significantly different (minimum possible value of ε)	Methyl Alcoholic C	0·297 0·297
5. Concerted reaction	Methyl Alcoholic C	0·61 0·44

there will be little change in the stereochemistry of the 2-carbon atom during the initial part of the reaction.   Large isotope effects will then be possible only in stretching, rupture, and formation of bonds attached to the labelled carbon atom.   In particular, no isotope effect will be obtained with methyl-labelled pinacol in three of every four molecules which react, since these will not involve a labelled carbon atom.

(2) *Transition State Unhydrated*.   If the transition state is an unhydrated synartetic ion of the type

$$
\begin{array}{c}
\quad\quad\quad CH_3 \\
CH_3\diagdown \quad\quad + \\
\quad\quad C{-}C{-}CH_3, \\
CH_3\diagup \quad\quad | \\
\quad\quad\quad\quad OH
\end{array}
$$

the whole carbon skeleton will be distorted from an ethane-type structure.   We might expect therefore that the vibration frequencies of the molecule will be affected even if a non-labelled carbon atom migrates.   Isotope effects will then be possible in stages III and IV in other labelled positions.   Two possibilities exist.   Firstly, the vibration frequencies of those bonds connected only to the alcoholic carbon atom which forms the carbonium ion (2-position) are altered. Secondly, the skeletal vibration frequencies of the entire molecule are affected. These alternatives are considered separately in Tables 12 and 13.

(3) *Concerted Reaction*.   If stages II, III, and IV are all slow and con- current, it is reasonable to enquire whether they proceed through three separate transition steps, or a single step.   If the latter, the transition step will be of the form

$$
\begin{array}{c}
\quad\quad\quad CH_3 \\
CH_3\diagdown \quad\quad + \\
\quad\quad C{-}C{-}CH_3. \\
CH_3\diagup \diagdown\; | \\
\quad\quad : \quad | \\
\quad\quad OH_2 \; OH
\end{array}
$$

Double isotope effects are possible with the 2-, 3-, and 4-carbon atoms, but in all other cases a single isotope effect may be possible, but only if the skeletal vibration frequencies are substantially different from those of the pinacol conjugate acid.

(iii) *Estimation of Maximum Isotope Effect*.—It is not possible to make any reliable estimate of the overall isotope effects experimentally expected.   We can, however, estimate the maximum possible isotope effect, from Bigeleisen's (1949) conclusion that for ^{14}C and ^{12}C the maximum difference in rate constant (assuming the labelled carbon atom to be completely free in the transition state) is given by

$$\varepsilon = k^*/k = \tfrac{2}{3},$$

where k is the rate constant of the isotopically normal molecule and k^* that of the molecule containing the heavy isotope. We have therefore proceeded by the following steps :

(1) The maximum possible isotope effect (minimum value of ε) has been set equal to

$$\varepsilon_{Total}=\frac{1}{\nu}\Sigma(\varepsilon_2)_n(\varepsilon_3)_n(\varepsilon_4)_n,$$

where $n=1, 4, 5, 6$; $\nu=4$ for methyl-labelled pinacol, and $n=2, 3$; $\nu=2$ for alcoholic carbon-labelled pinacol ; n refers to the labelling position.

(2) When no isotope effect is expected $(\varepsilon_m)_n$ is set equal to unity. When an equilibrium exists (i.e. in stage II) $(\varepsilon_2)_n$ is arbitrarily assumed to be $0\cdot9$. We have no means of estimating its exact value, although we should expect it to be nearer $0\cdot98$. The value $0\cdot9$ was chosen as the worst possible case. For kinetic isotope effects, $(\varepsilon_m)_n$ is given its minimum possible value of $\frac{2}{3}$.

(3) An intramolecular isotope effect is possible by competitive migration of the 4- and 6-methyl groups. This is estimated by the equation

$$\varepsilon_I=\frac{k^*}{k}=\left(\frac{\mu}{\mu^*}\right)^{\frac{1}{2}},$$

where the μ's are reduced masses, given by

$$\frac{1}{\mu}=\frac{1}{3+m_C}+\frac{1}{103},$$

where m_C is the mass of the carbon atom. Hence $\varepsilon_I=0\cdot96$. This estimate will not be greatly in error.

(iv) *Conclusion for High Temperature Reaction.*—The results of the calculation are shown in Table 13. Experimental values of ε_{exp} for methyl-^{14}C and alcoholic-^{14}C labelling are seen from Table 11 to be about $0\cdot49$ and $0\cdot73$ respectively. It thus follows that in Table 12 conditions 1, 2 (*a*), 3 (*a*), and especially 5 (the concerted reaction) are excluded. Condition 2 (*b*)—the assumption of maximum isotope effects in III and IV, with equilibrium in II—is theoretically possible. But since we have to assume minimum values of ε for all labelled positions, it is unrealistic. It is equivalent to assuming that all carbon atoms in stages III and IV are free and unbound to other carbon atoms. Condition 4 (*a*) (II not an equilibrium, but some restrictions in III and IV) and condition 4 (*b*) (minimum values of $(\varepsilon_m)_n$ in all steps) give one enough margin for either to be valid, although clearly neither can be accepted as reality.

It is, of course, possible that the reaction is much more complicated than we suspect, and certainly the results at lower temperatures lend some support to this belief. But if we accept that the high temperature reaction proceeds solely via the acid-base pre-equilibrium and the synartetic ion the following conclusions are inescapable :

(1) Stage II is not an equilibrium, although oxygen exchange equilibrium between water and pinacol is established. Pinacol rearrangement requires a larger extension of the C—O bond than is necessary for oxygen exchange.

(2) Stages II, III, and IV are sufficiently distinct for them to be regarded as requiring separate transition states. This gives a reasonable explanation of the high entropy of activation (36 cal/degree) which now is seen to be made up from contributions from three reactions. It also requires that the species

$$CH_3 \quad C^+ \!\!-\!\! C \quad CH_3 \qquad \text{and} \qquad CH_3 \quad C \!\!-\!\! C \!\!-\!\! CH_3$$

(chemical structures)
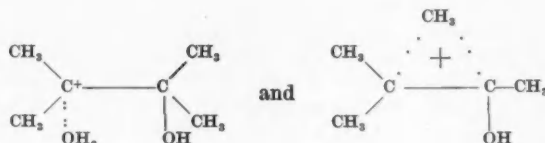
have transitory existence, although it does not imply that they are stable ions in the ordinary sense.

(3) Formation of the transition state involves all carbon atoms. It is already known that oxygen exchange of *sec.*-butanol involves stereochemical inversion of the carbon atom attached to the hydroxyl group (Bunton, Konasicwicz, and Llewellyn, 1955). If this is also a feature of the pinacol-pinacone rearrangement, it would provide an adequate explanation of the participation of the 1- and 5-carbon atoms in the formation of the transition state.

(4) The synartetic ion is unhydrated. Whilst the carbonium ion is not unhydrated, the water is bound only very loosely to it.

(c) *Interpretation of Results at Low Temperatures*

It is seen from Table 11 that no isotope effect is observable at low temperatures. This at once confirms that a second reaction mechanism is operative at low temperatures, a fact which also follows from the different kinetic equations obtained at these temperatures (Duncan and Lynn 1957). Further, we must conclude that those features leading to large isotope effects at high temperatures must either be absent in the low temperature mechanism or their effect must be eliminated by exchange reactions. The latter possibility seems unlikely. If the large isotope effects at higher temperature are real they are due to (i) the formation of an electronically unsaturated transition state which caused sufficient distortion in the carbon skeleton for *all* labelled carbon atoms to affect the rate of transport of the migrating group and (ii) the possibility of two or three stages, each of which leads to an isotope effect. Although there may be a small isotope effect at the low temperatures, it is less than 2 per cent. As shown in Tables 12 and 13, either of the above features could cause an isotope effect much larger than this. If both features are to be absent, the transition step in the low temperature reaction must be an uncharged species with all except the migrating methyl group maintained in a stereochemical state closely similar to that in its immediate precursor. A cyclic compound, such as an epoxide, would be a possible intermediate through which this reaction could proceed. But there are other possibilities, which will be discussed in detail at another time.

(d) *Interpretation of Results at Intermediate Temperatures*

At 78 °C the isotope effect is only obtained from measurements made on pinacol, and not from measurements on pinacone.   Reference to kinetic experiments made earlier (Duncan and Lynn 1956) will show that at this temperature the dependence of rate on [H⁺] is reasonably of first order, although the reaction has begun to show some deviations in the direction of a higher power dependence on [H⁺].   This shows the high temperature mechanism to be predominant at 78 °C, although there is a significant contribution from the low temperature mechanism.   Two alternative simultaneous reactions would not necessarily explain the results.   The several possibilities will be discussed in detail, in relation to other evidence in subsequent papers.

V. Acknowledgments

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THE STRETCHING FREQUENCY OF THE AZO–GROUP

By R. J. W. Le Fèvre* and R. L. Werner†

Summary

Infra-red absorptions now reported for 25 azoaryl derivatives bring the number of $N=N$- containing structures examined so far up to 83. Indications previously recognized, that the azo-stretching frequencies in azobenzenes lie between 1400 and 1450 cm^{-1}, are confirmed by the present measurements.

I. INTRODUCTION

A survey of the infra-red spectra of 43 substances, believed to contain the $-N=N-$ group, made by Le Fèvre, O'Dwyer, and Werner (1953), led to the tentative conclusion that the azo-link was possibly responsible for either or both of the common absorptions found around 1400 and 1580 cm^{-1}. A later examination of 10 formazans and 5 copper chelates therefrom (Le Fèvre, Sousa, and Werner 1955) tended to support the former alternative, since the band at *c.* 1450 cm^{-1} in each of the formazans was destroyed by chelation (a process in which one of the azo-nitrogens coordinates as a donor to the metal atom) while that at *c.* 1580 cm^{-1} persisted.

We now desire to record data for a further 25 compounds, mainly derivatives of azobenzene, prepared in recent years for other purposes by Professor G. M. Badger and collaborators in the laboratories of the University of Adelaide.

II. EXPERIMENTAL

Details are as stated in Le Fèvre, Sousa, and Werner's (1955) paper. The spectra were determined on the solid specimens mulled in " Nujol " or hexachlorobutadiene. Tables 1 to 4 present data in " Nujol ". Table 5 considers three cases in which the bands due to " Nujol " obscured features revealed by the use of hexachlorobutadiene. Intensities are indicated relatively to the strongest absorptions in a given spectrum as strong (s), medium (m), and weak (w). The preparations of most of the specimens are described by Badger and Lewis (1953*a*, 1953*b*).

* School of Chemistry, University of Sydney.
† School of Applied Chemistry, N.S.W. University of Technology, Broadway, Sydney.

TABLE 1

DERIVATIVES OF AZOBENZENE

4-Me	4-F	4-Cl	4-Br	4-I	4-(NO$_2$)	4-(CO$_2$H)	4-(OH)	4-Hydroxy-2',3-azo-toluene	4-(OMe)	4-(SCN)
										2162 (m)
										1662 (w)
						1678 (s)				
1607 (w)	1595 (s)	1591 (m)	1582 (m)	1579 (w)	1605 (m)	1607 (m)	1605 (s)	1612 (m)	1605 (s)	1591 (w)
1587 (w)	1586 (s)	1579 (m)	1576 (m)	1568 (m)	1585 (m)	1586 (m)	1587 (s)	1591 (ms)	1588 (s)	1577 (w)
1506 (w)	1503 (s)				1518 (ms)	1503 (w)	1507 (ms)	1507 (m)	1503 (s)	
1488 (m)	1482 (s)	1483 (s)	1477 (s)			1488 (w)		1484 (m)		
									1455 (s)	
1448 (s)	1448 (s)	1445 (ms)	1458 (s)	1442 (m)	1442 (ms)	1445 (ms)			1448 (s)	1445 (ms)
						1431 (s)				
1411 (w)	1409 (w)	1402 (m)	1378 (s)	1392 (m)	1409 (w)	1406 (m)		1412 (m)	1417 (w)	1403 (m)
					1336 (s)			1357 (m)	1323 (w)	
1300 (m)	1301 (w)	1295 (m)	1304 (w)	1293 (m)	1310 (s)	1310 (s)			1304 (m)	1300 (m)
			1293 (w)							
		1275 (w)	1277 (w)	1275 (w)	1274 (m)	1287 (s)	1275 (s)	1272 (s)	1295 (m)	1282 (w)
							1237 (s)	1252 (s)	1248 (s)	
1220 (w)	1226 (s)	1218 (w)	1218 (w)	1218 (w)		1220 (m)	1220 (s)			1221 (w)
1209 (w)		1211 (w)			1211 (w)					
					1172 (w)	1184 (w)		1179 (m)		
1151 (s)	1161 (w)		1161 (w)	1152 (ms)	1160 (w)	1158 (w)	1156 (m)	1156 (m)	1152 (m)	1158 (w)
	1145 (m)	1149 (s)	1149 (m)			1144 (w)	1142 (s)		1145 (m)	
	1136 (ms)					1127 (w)				
						1111 (w)		1115 (w)		
1109 (m)	1094 (m)	1100'(m)	1098 (w)	1101 (m)	1105 (m)	1102 (w)			1106 (ms)	1105 (m)
	1087 (s)	1087 (s)			1089 (w)			1093 (ms)		
1070 (w)	1070 (w)	1071 (m)	1070 (ms)	1067 (w)	1068 (w)	1071 (w)			1070 (ms)	1071 (ms)
							c. 1080 (m)			
1035 (w)										
1017 (w)								1040 (m)	1028 (s)	
1012 (w)	1018 (w)	1018 (w)	1018 (w)	1016 (w)	1023 (w)	1012 (m)				1008 (w)
		1006 (m)	1009 (m)		1003 (w)					
	1000 (w)	1000 (w)	1004 (ms)	999 (s)		1000 (w)	998 (w)	1000 (w)		
						948 (s)	935 (w)	957 (m)	945 (w)	
921 (w)	921 (w)	918 (m)	915 (m)	915 (w)	911 (w)	921 (s)	926 (w)		925 (m)	929 (w)
							917 (w)		918 (m)	
					859 (s)		903 (w)			
							890 (ms)			
839 (m)	843 (s)	840 (s)	836 (s)	840 (s)		841 (w)	835 (w)		838 (s)	831 (s)
820 (s)	819 (m)	822 (m)	822 (ms)				804 (w)	818 (s)	825 (s)	
									814 (w)	
	778 (ms)				777 (ms)	788 (m)	790 (w)			
						780 (s)				
		770 (ms)	769 (m)						772 (s)	770 (s)
762 (s)	766 (s)	764 (ms)	763 (m)	769 (s)	760 (m)	763 (w)	764 (m)	762 (s)	766 (s)	
					754 (s)				759 (s)	
		726 (w)	722 (w)	722 (w)	722 (w)	722 (m)	719 (m)	723 (m)	722 (s)	
710 (s)	714 (ms)	710 (ms)	706 (m)	703 (ms)	697 (ms)	695 (s)				710 (ms)
										704 (m)
										694 (m)
						687 (ms)			687 (s)	
685 (s)	684 (s)	680 (s)	685 (s)	681 (s)	683 (s)	684 (ms)	681 (s)			686 (s)

TABLE 2

DERIVATIVES OF 4-AMINOAZOBENZENE

4-(NHMe)	4-(NMe$_2$) -4'-(NO$_2$)	4-(NMeEt)	4-(NMe. CH$_2$C$_6$H$_5$)	4-N(Me. CH$_2$CH$_2$OH)	4-(NEt$_2$)
1600 (s)	1603 (s)	1602 (s)	1602 (s)	1604 (s)	1604 (s)
1583 (s)	1589 (s)				
	1552 (m)	1564 (ms)	1563 (m)	1563 (m)	1566 (m)
1531 (ms)	1515 (s)	1518 (s)	1518 (s)	1519 (ms)	1515 (s)
1503 (w)			1497 (m)		
	1448 (m)				
1408 (s)	1425 (m)	1412 (ms)	1412 (m)	1406 (m)	1403 (s)
1336 (s)	1331 (s)	1343 (ms)	1353 (ms)	1352 (ms)	1360 (s)
		1314 (ms)	1314 (m)	1313 (ms)	1312 (m)
1305 (m)	1305 (s)			1301 (ms)	1300 (m)
1286 (m)	1290 (s)				1282 (m)
		1270 (ms)			1264 (s)
1243 (m)	1254 (ms)	1246 (ms)	1255 (m)	1249 (m)	1264 (ms)
	1232 (ms)				
		1215 (ms)	1209 (s)	1205 (ms)	
	1197 (m)				1196 (s)
	1176 (m)				
1155 (m)	1153 (ms)	1162 (ms)	1159 (s)	1158 (ms)	1153 (s)
1136 (s)	1135 (s)	1142 (s)	1142 (s)	1147 (ms)	1139 (s)
			1123 (ms)	1129 (ms)	1119 (s)
	1103 (s)	1086 (ms)	1113 (ms)	1112 (ms)	1094 (s)
1071 (w)	1066 (s)	1067 (ms)	1068 (w)	1068 (s)	1066 (s)
1020 (w)		997 (m)	1021 (w)		1015 (m)
962 (w)		984 (ms)	1004 (w)	974 (w)	
			965 (w)	951 (w)	
	941 (m)	937 (ms)	949 (m)	932 (w)	
			942 (m)		
920 (w)		910 (ms)	906 (w)	900 (w)	918 (w)
	862 (s)			864 (w)	
	857 (s)				
830 (m)	827 (s)	815 (s)	815 (s)	822 (s)	824 (s)
822 (m)		815 (s)	815 (s)	822 (s)	824 (s)
		797 (m)	790 (w)		788 (m)
		789 (m)			
766 (m)	755 (m)	767 (s)	766 (ms)	771 (s)	764 (s)
			746 (w)		
	737 (w)		731 (s)		
723 (w)	729 (w)	723 (m)	721 (ms)	725 (m)	724 (ms)
690 (m)	690 (m)	691 (s)	696 (ms)	692 (ms)	690 (s)
			688 (s)		

TABLE 3

META-DERIVATIVES OF AZOBENZENE

3-F	3-Cl	3-Br	3-(NO$_2$)	3-(COOH)
				1680 (s) broad
			1611 (w)	1602 (m)
1594 (s)	1587 (w)	1586 (m)	1588 (w)	1598 (s)
	1575 (w)	1569 (m)		
			1521 (s)	1534 (w)
			1482 (s)	1496 (w)
1450 (s)	c. 1460 (s) broad	1456 (s)		1455 (s)
		1448 (s)	1449 (s)	
1428 (m)		1409 (w)		1426 (ms)
			1355 (s)	
			1343 (s)	
			1321 (ms)	1327 (s)
1308 (m)	1301 (w)	1304 (w)	1304 (ms)	1307 (s)
1271 (w)				1275 (s)
1249 (ms)				
1238 (ms)	1206 (m)	1207 (ms)	1213 (m)	1214 (m
1192 (m)				1181 (w)
	1169 (m)	1170 (m)		1161 (m)
1151 (w)	1147 (m)	1148 (m)	1146 (m)	1146 (w)
1111 (ms)		1135 (w)	1136 (m)	1106 (w)
		1083 (w)		1093 (m)
1071 (ms)	1068 (m)	1071 (w)	1073 (m)	1078 (m)
				1070 (m)
		1056 (w)	1066 (m)	
1018 (w)	1018 (w)	1018 (w)	1018 (m)	1019 (w)
1002 (w)	995 (w)	993 (w)	1000 (m)	998 (m)
974 (m)				968 (m)
	931 (m)			936 (s)
924 (m)	924 (m)	928 (m)	922 (m)	
	912 (m)	912 (m)	909 (m)	918 (s)
888 (s)	881 (ms)	881 (ms)	877 (w)	867 (w)
878 (s)				
	859 (m)			
	849 (ms)	840 (ms)	814 (s)	820 (s)
792 (s)	788 (s)	789 (s)		785 (m)
765 (s)	765 (s)	767 (s)	770 (s)	762 (s)
	720 (w)		739 (ms)	720 (m)
	713 (w)		707 (w)	
690 (s)	689 (s)	688 (s)	698 (w)	
680 (m)		681 (ms)	685 (s)	684 (s)
			666 (m)	

TABLE 4

AZO-HYDROCARBONS

Phenylazo-α-naphthalene	Phenylazo-β-naphthalene	αβ'-Azo-naphthalene
	1628 (w)	1630 (w)
1591 (w)	1597 (w)	1591 (w)
1575 (w)		1572 (w)
1511 (w)		1507 (m)
1473 (m)	1431 (m)	
1346 (w)	1354 (m)	1346 (m)
c. 1300 (w) broad	1300 (w)	
	1266 (w)	1264 (w)
c. 1250 (vw)	1245 (w)	1245 (w)
1215 (m)	1212 (w)	1210 (w)
c. 1180 (vw)		1196 (w)
1156 (w)	1164 (m)	1159 (w)
	1158 (w)	
1141 (w)	1143 (w)	1142 (w)
	1109 (w)	1111 (m)
1071 (w)	1072 (m)	1082 (w)
1021 (w)	1019 (w)	1011 (w)
1013 (w)		
	952 (ms)	954 (w)
923 (ms)	927 (ms)	
	893 (s)	898 (ms)
871 (w)	863 (ms)	861 (ms)
842 (w)		827 (w)
	820 (s)	819 (ms)
804 (s)		806 (ms)
781 (m)	771 (m)	772 (s)
	767 (m)	
761 (s)	760 (m)	756 (ms)
735 (w)	747 (s)	
687 (s)	689 (s)	677 (w)

TABLE 5

PORTIONS OF THE PREVIOUS SPECTRA REPEATED WITH HEXA-
CHLOROBUTADIENE AS MULLING AGENT

Phenylazo-α-naphthalene	αβ′-Azo-naphthalene	3-Chloroazo-benzene
1510 (m)	1506 (m)	
1496 (w)		
1483 (ms)		1487 (ms)
	1477 (w)	1475 (ms)
1470 (w)		1472 (ms)
1457 (w)	1459 (w)	1456 (s)
		1446 (s)
1433 (w)		1424 (m)
		1412 (m)
1389 (s)	1389 (w)	
1377 (m)	1381 (w)	
	1358 (w)	
	1352 (w)	
1346 (ms)	1345 (m)	
	1322 (w)	
1306 (w)		1306 (m)
1301 (w)		

III. DISCUSSION

A review of Tables 1 to 5 reveals 10 regions within the limits of which common absorptions occur. All except one can, on present knowledge, be credibly assigned to links or structural units other than the azo-group (cf. Bellamy 1954) as shown in Table 6.

TABLE 6

SUGGESTED ASSIGNMENTS OF COMMON ABSORPTIONS

Region (cm^{-1})	Assignment	Region (cm^{-1})	Assignment
1564–1612	" Benzenoid bands "	1050–1080	ν_{14} of benzene
1400–1460	?	903–957	C–N
1264–1314	C–N	815–878	1,4-$C_6H_4X_2$
1206–1252	Substituted Ar	755–771	C–H (out-of-plane)
1142–1164	C–N	677–729	C–H (out-of-plane)*

* In one case in Table 1 (4-hydroxy-2′,3-azotoluene) the strong band at c. 680 cm^{-1} is absent. This is to be expected for a molecule lacking a mono-substituted phenyl group (cf. Bellamy 1954, p. 65).

As with the formazans of Le Fèvre, Sousa, and Werner (1955) the recurring frequencies between 1400 and 1460 cm^{-1} remain unexplained. Bellamy (1954, p. 221) notes that NMe in simple molecules may produce effects at 1430–1460 cm^{-1} and that (unnamed) " more complex materials " often show

weak absorption near 1430 cm^{-1}. However, among the present spectra the region 1400–1460 cm^{-1} is usually occupied by at least one band which is not " weak " :

para-Derivatives of Azobenzene

p-Me	1448 (s)	p-NHMe	1408 (s)
p-F	1448 (s)	p-NMeEt	1412 (ms)
p-Cl	1445 (ms)	p-NMe.CH$_2$C$_6$H$_5$	1412 (ms)
p-Br	1458 (s)	p-NMe.CH$_2$CH$_2$OH	1406 (m)
p-I	1442 (m)	p-NEt$_2$	1403 (s)
p-NO$_2$	1442 (ms)	p-NMe$_2$-p'-NO$_2$	$\begin{cases} 1448 \text{ (m)} \\ 1425 \text{ (m)} \end{cases}$
p-CO$_2$H	1431 (s)	p-HO	1419 (ms)
p-MeO	$\begin{cases} 1455 \text{ (s)} \\ 1448 \text{ (s)} \end{cases}$	p-SCN	1445 (ms)

meta-Derivatives of Azobenzene

m-F	$\begin{cases} 1450 \text{ (s)} \\ 1428 \text{ (m)} \end{cases}$	m-Br	$\begin{cases} 1456 \text{ (s)} \\ 1448 \text{ (s)} \end{cases}$
m-Cl	$\begin{cases} 1456 \text{ (s)} \\ 1446 \text{ (s)} \end{cases}$	m-NO$_2$	1449 (s)
		m-CO$_2$H	1455 (s)
4-Hydroxy-2′,3-azotoluene			1412 (m)

Naphthalene Derivatives

Phenylazo-α	$\begin{cases} 1457 \text{ (w)} \\ 1433 \text{ (w)} \end{cases}$	Phenylazo-β-	1431 (m)
		$\alpha\beta'$-Azonaphthalene	1459 (w)

Accordingly, the results now recorded reinforce suspicions that the N=N stretching frequency in the azobenzenes is located near 1450 cm^{-1}, and the fact that substituents such as NR$_2$, OH, CO$_2$H in positions *para* to the azo-link cause a lowering of this frequency is consistent with expectations based on resonance.

IV. CONCLUSIONS

(i) The suggestion just mentioned, namely, that $\nu_{N=N}$ lies between 1400 and 1450 cm^{-1} in the simpler azoaryls, is the only assignment satisfactory for the 83 N=N-containing molecules studied to date in this Laboratory.

(ii) In the light of present data, the conclusion of Le Fèvre, Sousa, and Werner (1955), that in formazans absorption at *c.* 1450 cm^{-1} is characteristic of −N=N− modified by conjugation and hydrogen bonding, should probably be amended by deleting the reference to hydrogen bonding.

V. ACKNOWLEDGMENT

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THE PERMANGANATE–COLOURED " DIMER " OF DIPHENYLKETEN

By R. S. Armstrong* and R. J. W. Le Fèvre*

Summary

The substance $C_{28}H_{20}O_2$ formed by the pyrolysis of benzilic acid, has a dipole moment of *c.* 6 D and shows ketonic absorptions in the infra-red spectral region. These observations can be harmonized with views stated by Schönberg and Sina (1947).

I. Introduction

The present paper considers a crystalline substance, with the appearance of a metallic permanganate, obtained in small yield by the thermal decomposition of benzilic acid in the presence of anhydrous sodium carbonate or potassium benzilate (Langenbeck and Langenbeck 1928). The material showed the same composition, namely, $C_{28}H_{20}O_2$, as dimeric diphenylketen. Since Staudinger (1911) had already described a colourless dimeric isomer to which a 1,3-*cyclo*butadione skeleton (I*a*) was applicable, the Langenbecks represented the new compound by structure I.
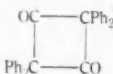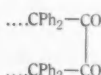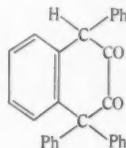
(I)	(I*a*)	(II)	(III)

Determination of structure by chemical methods was made difficult by the resinification induced by the most varied reagents. With ethanolic potassium hydroxide or cyanide, phosphorus pentachloride, sulphonyl chloride, bromine, or phenylhydrazine, no crystalline derivative could be isolated. Chromic oxide oxidation gave 43 per cent. of the benzophenone (2 mol) expected on the basis of I.

Wittig and Lupin (1928) challenged I on the grounds that the substance lacked reactions characteristic of 1,2-cyclic diketones ; they suggested the *di*radical formula II. This was opposed by Müller (1935), who found the solid to be not paramagnetic. Although Allen and Sugden (1936) remarked in another connection that the magnetic test was not necessarily decisive for

* School of Chemistry, University of Sydney.

*di*radicals, the stability of the compound towards oxygen strongly supported the rejection of II.   Schlenk (1939) accordingly advanced III, although without any fresh experimental evidence.

Criticism of III has come from Schönberg and Sina (1947), who have replaced it by IV, IV*a*, or IV*b*, with the possibility of resonance between the three.

$$PH_2C \quad O \qquad PH_2C \quad O \qquad \overset{+}{PH_2C} \quad \overset{-}{O} \qquad PH_2C$$

(IV)   (IVa)   (IVb)   (V)
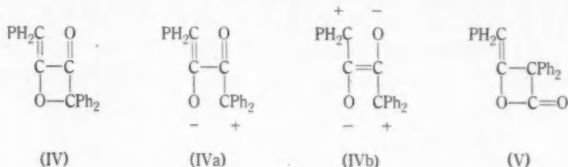
There seems no reason why V, the tetraphenyl analogue of dimeric keten (cf. Whiffen and Thompson 1946), should not be considered as well.

II. Present Work

During the past 8 years the Langenbeck's product has been prepared and examined on a number of occasions in these Laboratories by Miss I. Youhotsky, Mr. K. M. C. Mostyn, and the present authors.   Attempts to improve the yield, by varying the experimental conditions, by adding substances, e.g. benzophenone, benzoin, benzil, benzilide, etc. which might play the role of intermediates, have not been successful.   The unsatisfactory results of the chemical reactions mentioned above have been confirmed.   However, some new physical data have been accumulated, and these will now be reported since they may be useful in the eventual assignment of a structure to this curious compound.

III. Experimental
(a) Preparation of " Dimer "

Benzilic acid (20 g) and anhydrous sodium carbonate (0·2 g) were thoroughly ground and mixed, placed in a small flask (plus air condenser), and heated strongly for a few minutes over a bare flame.   Brownish red fumes were emitted and a dark purplish red viscous liquid remained, which cooling failed to solidify.   The sticky mass was shaken with strong aqueous sodium carbonate to remove most of the excess acid.   Solution and solid were then separated, and the latter taken up in carbon tetrachloride (100 ml), again shaken with sodium carbonate solution, and dried (calcium chloride).   Partial evaporation afforded a crystalline deposit, m.p. 158–162 °C, repeated crystallization of which from ethyl acetate gave ultimately 0·3 g, m.p. 169 °C.   For use in spectral work, the dimer was further purified by chromatography of the carbon tetrachloride solution on an alumina column, with benzene as eluting agent.

(b) Apparatus and Methods

Dielectric constants were determined with the apparatus described by Buckingham *et al.* (1956).   Other measurements, and calculations of polarizations, moments, etc. are as reported by Le Fèvre (1953).   Infra-red spectra

<div style="text-align:center">

TABLE 1

DIELECTRIC POLARIZATIONS OF " DIMER " IN BENZENE

</div>

$10^6 w_2$	ε	d_4^{25}
0	2·2725	0·87378
699·3	2·2793	0·87397₅
898·4	—	0·87399
959·0	2·2821	—
965·2	2·2818	0·87405
972·0	2·2822	—
1002·0	2·2832	0·87401
1120·7	2·2838	0·87405
1215·5	2·2844	0·87406₅
1824·0	2·2911	0·87421₅
2272·0	2·2974	0·87437
2581·0	2·3003	0·87448₅

Whence $\Sigma\Delta\varepsilon/\Sigma w_2 = 10\cdot2\pm0\cdot5 = \alpha\varepsilon_1$,
$\Sigma\Delta d/\Sigma w_2 = 0\cdot25\pm0\cdot02 = \beta d_1$,
and $_\infty P_2 = _\infty(p_2)M_2 = [p_1(1-\beta)+C\alpha\varepsilon_1]M_2 = 854\pm40$ c.c.
$p_1 = 0\cdot34086$ c.c. and $C = 0\cdot18809$ (cf. Le Fèvre 1953)
Taking $(R_2)_D$ as 124 c.c. (i.e. $2\times R_{Ph_2C:C:O}$) the dipole
moment of the " dimer " is $6\cdot0\pm0\cdot3$ D

<div style="text-align:center">

TABLE 2

SPECTROSCOPIC DATA

</div>

Infra-red absorption (cm⁻¹)* of the " dimer " as a mull of " Nujol "	1742 (vs) 1729 (vs) 1705 (m) 1657 (vw) 1638 (vw) 1586 (w) 1569 (vw) 1534 (m)	1364 (m) 1318 (w) 1300 (m) 1266 (w) 1222 (m) 1205 (vw) 1199 (w) 1149 (w)	1079 (w) 1058 (vw) 1028 (w) 1000 (vw) 960 (m) 936 (m) 906 (w) 877 (m)	818 (m) 776 (s) 761 (m) 751 (w) 709 (m) 705 (m) 691 (vs)

Infra-red absorptions (cm⁻¹)† of the " dimer " dissolved in pyridine	1750 (vs)	1732 (vs)

Ultraviolet absorptions (cm⁻¹)‡ of the " dimer " dissolved in cyclo-hexane or dioxan		$\lambda_{max.}$ $(m\mu)$	$\varepsilon_{max.}$
	In cyclohexane	283 458	15100 40800
	In dioxan	284 463	13800 36300

* s, strong ; m, medium ; w, weak ; vs, very strong ; vw, very weak.

† Further bands were not investigated, as disentanglement from the spectrum of pyridine was difficult.

‡ The spectra were simple, with two maxima in each solvent.

were [...]
Werne[...]
Beckm[...]

T[...]
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(cf. W[...]

A[...]
μ_{CPh_2O}[...]
upon [...]
Theref[...]
clear [...]

were recorded with the double-beam instrument constructed by Mr. R. L. Werner in the N.S.W. University of Technology, ultraviolet absorptions with a Beckman DU photoelectric quartz spectrophotometer.

(c) Measurements

Table 1 gives the observed dielectric constants (ε), and densities (d_4^{25}) for solutions of weight fraction w_2 in benzene at 25 °C (subscripts 1 and 2 indicate solvent and solute respectively). The total polarization of the " dimer " at infinite dilution is evaluated as presented in Table 1. Spectroscopic data are presented in Table 2.

IV. DISCUSSION

Moments may be anticipated for structures I to V by analogy as presented in Table 3. Structures IV, IVa, and IVb were advanced by Schönberg and Sina

TABLE 3

MOMENTS FOR STRUCTURES I TO V

Structure	Order of μ Expected (D)	Basis of Estimate
I or III	5–6	Phenanthraquinone, $\mu=5\cdot6$, or acenaphthaquinone, $\mu=6\cdot1$ (Caldwell and Le Fèvre 1939)
II	c. 4	Benzil, $\mu=3\cdot8$ (Caldwell and Le Fèvre 1939)
IV	2–4	Xanthone, $\mu=3\cdot1$ (Le Fèvre and Le Fèvre 1937a) ; 2,6-diphenyl-3,5-dimethyltetrahydro-γ-pyrone, $\mu=1\cdot8$; 2,6-diphenyl-γ-pyrone, $\mu=3\cdot8$ (Le Fèvre and Le Fèvre 1937b)
V	3–5	Dimeric keten, $\mu=3\cdot3$ (Angus et al. 1935) ; diphenylmethyleneanthrone, $\mu=3\cdot8$ (Le Fèvre and Youhotsky 1953) ; coumarin, $\mu=4\cdot5$ (Le Fèvre and Le Fèvre 1937b) ; γ-butyrolactone, $\mu=4\cdot1$ (Marsden and Sutton 1937)

as contributors to a hybrid real form. Through symmetry, IVb should be non-polar, while IVa, as a C-enol betaine, might have a moment around 10 D (cf. Wyman 1936).

Although replacement of H by C_6H_5 often raises polarity (e.g. $\mu_{CH_2O}=2\cdot1$, $\mu_{CPh_2O}=2\cdot95$; $\mu_{CH_2:CO}=1\cdot43$, $\mu_{CPh_2:CO}=1\cdot76$), this effect cannot be depended upon (e.g. $\mu_{coumarin}=4\cdot5$, $\mu_{3\text{-phenylcoumarin}}=4\cdot3$ D (Le Fèvre and Le Fèvre 1937b). Therefore, the moment now found (6 D) for the " dimer " does not permit a clear choice from among the possibilities I to V.

However, as already stated, II can be dismissed on chemical (if not also on magnetic) grounds. Non-formation of quinoxalines with 1,2-diamines was held by Wittig and Lupin to be against I; the same objection is valid with III, unless steric hindrance be invoked to explain the situation. It is relevant that 1-phenylindane-2,3-dione (VI) *does* form a quinoxaline, and in the
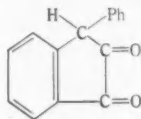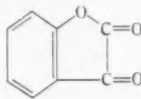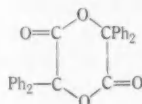
(VI)   (VII)   (VIII)

crystalline state closely resembles potassium permanganate (Koelsch 1936); moreover, it is easily enolized and dissolves in aqueous sodium hydroxide to blue solutions. In the last-named respects the " dimer " is in contrast to VI. Rejection of I, II, and III leaves only IV and V, or resonant modifications of them.

The infra-red spectrum of the " dimer " as a solid or as a solute in pyridine shows two strong ketonic bands at 1740–1750 and 1730 cm^{-1}. These are close to the lower carbonyl frequency observed by Grove (1951) with coumarin-2,3-dione (VII), namely, 1740 cm^{-1}, and near to that listed by Grove and Willis (1951) for C=O in phthalide rings and certain cyclic lactones. The relatively unstrained 6-ring benzilide (VIII), presumed to be an intermediate in the formation of the " dimer ", has a single band between 1760 and 1770 cm^{-1} (Wasserman and Zimmerman 1950). In VII the " lactone carbonyl " absorbs at 1833 cm^{-1}; the " dimer ", however, shows no such high-frequency absorption. Spectral evidence thus indirectly tends to disfavour the β-lactone formula V, and also an alternative with the O and CO interchanged; β-butyrolactone displays its C=O group at 1818 cm^{-1} (Taufen and Murray 1945), and in the light of the remarks of Grove and Willis a frequency *c.* 1800 cm^{-1} is not an unreasonable expectation for a 4-ring lactone.

There remain therefore formulae based on IV. In saturated ketones $\nu_{C=O}$ rises with increased ring strain : *cyclo*hexanone, 1710 cm^{-1}; *cyclo*pentanone, 1740 cm^{-1}; *cyclo*butanone, 1775 cm^{-1} (cf. Bellamy 1954); conjugation causes a lowering of the frequency, e.g. αβ-unsaturation to a C=O group diminishes $\nu_{C=O}$ by *c.* 40 cm^{-1} in both 5- and 6-membered rings (Le Fèvre, Maramba, and Werner 1953; cf. Bellamy 1954). Superimposition of these two effects in the case of IV may explain the absorptions now observed for the " dimer ".

It should be added that no signs of OH in the 3400 cm^{-1} region could be detected; this confirmed negative results obtained by Zerewitinoff determinations.

V. Conclusions

Structure IV seems least unacceptable. *Alone* it cannot be reconciled with the observed moment of 6 D since the polarity of the unit $Ph_2C=C-C=O$ could hardly exceed *c.* 4 D (cf. cinnamaldehyde, $\mu=3\cdot7$; or benzylidene-

acetophenone, $\mu = 2 \cdot 9$ D) and in the molecular resultant would be reduced by the " ether " component. Recourse must be had, therefore, to Schönberg and Sina's (1947) suggestion of mesomerism between IV and the " zwitterionic " IVa. The presence of a partially positive C atom adjacent to the carbonyl will diminish or prevent resonance towards $Ph_2C^+ - C = C - O^-$, and thus raise the ν_{CO} in the way now recognized for electropositive radicals situated α to a carbonyl group (cf. Bellamy 1954, p. 121).

VI. ACKNOWLEDGMENT

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REACTIONS OF AROYL PEROXIDES

I. BENZOYL PEROXIDE WITH BENZENE

By B. M. Lynch* and K. H. Pausacker†

[*Manuscript received October 2, 1956*]

Summary

A quantitative study of the products from the reaction of benzene with benzoyl peroxide, under varying conditions, is reported. The mechanisms of formation of the various products are discussed.

I. Introduction

The reactions of aromatic compounds with aroyl peroxides have been studied intensively during recent years by various workers (Dannley and Zaremsky 1955; Rondestvedt and Blanchard 1955, 1956; Hey, Stirling, and Williams 1956) but few attempts have been made to determine all the reaction products. In this series of papers, certain of these systems will be investigated in detail in an attempt to establish the complete stoichiometry of the reactions involved. The present paper reports the results obtained from a study of the reactions of benzene with benzoyl peroxide.

For this reaction system, the best available product study is that of Gelissen and Hermans (1925) who showed that reaction at 80 °C yielded benzoic acid, carbon dioxide, diphenyl, phenyl benzoate, and resinous products, together with small yields of *p*-terphenyl and 4,4′-diphenyldiphenyl (quaterphenyl). Dannley and Gregg (1954) have shown that reaction of benzoyl peroxide with an equimolar mixture of benzene and pyridine at 70 °C yields appreciable amounts of phenylbenzoic acids.

Barnett and Vaughan (1947) have investigated the effects of temperature and of peroxide concentration upon the yields of carbon dioxide and benzoic acid. At 80 °C, they found that the yield of carbon dioxide decreased from 1·55 to 1·13 mol‡ as the peroxide concentration was increased from 0·08–1·23M, and the yield of benzoic acid increased from 0·27–0·68 mol. Further, in 0·08M solution, as the temperature was raised from 55 to 80 °C, the yield of carbon dioxide increased from 0·72 to 1·55 mol, while that of benzoic acid decreased from 1·14 to 0·27 mol. Investigations by other workers (McClure, Cuthbertson, and Robertson 1942; Hartman, Sellers, and Turnbull 1947) support these results.

* Chemistry Department, New Mexico Highlands University.
† Chemistry Department, University of Melbourne.
‡ All yields are per mol of benzoyl peroxide.

II. Experimental

Analyses were made by the C.S.I.R.O. Microanalytical Laboratory.

(a) Reagents

Benzoyl peroxide (B.D.H.) was purified by double precipitation from chloroform with methanol (ice-cooling), and was dried *in vacuo* over phosphorus pentoxide. It has a minimum purity of 98 per cent. when estimated by the method suggested by Skellon and Wills (1948). Benzene was purified according to Vogel (1950). Benzoic acid was recrystallized from ethanol and had m.p. 122 °C. 2,4,6-Tribromophenol had m.p. 93 °C (from ethanol-water). Diphenyl (B.D.H.) and *p*-terphenyl (B.D.H.) were recrystallized, their m.p.'s being 70 °C (from ethanol) and 206 °C (from benzene).

(b) General Procedure

Reactions were carried out in a vapour-jacketed vessel connected to a gas-burette. The jacketing liquid (ethanol) was heated to slow reflux, and the appropriate volume of benzene added to the inner bulb. When temperature equilibrium was attained, the benzene was saturated with carbon dioxide. Benzoyl peroxide was added rapidly with stirring, and the system was closed. Heating was continued until gas evolution ceased ; the volume evolved, corrected for the partial vapour pressure of benzene, was recorded. Gelissen and Hermans (1925) have shown that the evolved gas consists entirely of carbon dioxide. The solid which separated on cooling was filtered off and weighed. It was identified as 4,4'-diphenyldiphenyl (quaterphenyl), after crystallization from benzene, by m.p. 312 °C (lit. 312 °C) (Found : C, 94·2 ; H, 6·2 per cent. Calc. for $C_{24}H_{18}$: C, 94·1 ; H, 5·9 per cent.), and ultraviolet absorption spectrum (light absorption in chloroform $(3·85 \times 10^{-5}M)$: max. at 2980 Å ($\varepsilon = 39000$) ; cf. Gillam and Hey 1939). Extraction of the filtrate with 5 per cent. sodium hydrogen carbonate, followed by acidification of the extract, gave benzoic acid (referred to as " 1st benzoic acid ") which was collected ; the yield, m.p., and neutralization equivalent were determined. Benzene in excess was removed from the residual reaction mixture under reduced pressure, and the residue was refluxed (8 hr) with 10 per cent. potassium hydroxide in ethanol (100 ml). The ethanol was removed under reduced pressure and the residue was extracted with ether and with water. A further quantity of 4,4'-diphenyldiphenyl separated and was filtered off and weighed. The ethereal layer was reserved (extract A), and the aqueous layer was acidified with dilute sulphuric acid and extracted with ether (extract B). Phenol (identified as 2,4,6-tribromophenol, m.p. and mixed m.p. 90–95 °C) and an additional quantity of benzoic acid (referred to as " 2nd benzoic acid "), m.p. and mixed m.p. 120–122 °C, were obtained from extract B by standard methods. Extract A was dried (Na_2SO_4) and the ether removed under reduced pressure. The residue was distilled under reduced pressure and a fraction, b.p. 90–92 °C/0·3 mm, was collected. It was identified as diphenyl (m.p. and mixed m.p. 69–70 °C). The material remaining in the distillation flask (referred to as " high-boiling residue ") was weighed and reserved.

(c) Results

The results obtained are summarized in Table 1. The values in parentheses are average yields in mol/mol of benzoyl peroxide. Details of investigations of the various high-boiling residues are given in the footnotes accompanying Table 1. Unless otherwise stated, reactions were carried out at 78 °C.

TABLE 1

PRODUCTS FORMED IN THE REACTION OF BENZOYL PEROXIDE WITH BENZENE

Experiment No. :	1*	2	3, 4	5	6, 7	8, 9	10†
$(Ph.CO_2)_2$ (g)	6	6	6	20	20	7	60
PhH (ml)	200	300	200	300	200	30	200
Reaction time (hr)	1560	27	23, 29	24	23, 23	12, 12	20
Vol. CO_2 (ml)	—	750	762	2340	2225	713	(1·14)
		(1·34)	745	(1·27)	2113	752	
			(1·36)		(1·19)	(1·19)	
1st $Ph.CO_2H$ (g)	1·36	1·28	1·31	4·40	4·50	2·15	17·2
	(0·45)	(0·42)	1·28	(0·44)	4·50	2·15	(0·57)
			(0·42)		(0·45)	(0·61)	
1st $Ph.CO_2H$ (m.p. °C)	120	120	121	114–116	114–115	99–102	—
1st $Ph.CO_2H$ (equiv.)	126	127	126	129	129	140	—
			126		129	141	
2nd $Ph.CO_2H$ (g)	0·69	0·73	0·78	3·00	2·80	—	6·0
	(0·23)	(0·24)	0·72	(0·30)	3·30		(0·20)
			(0·25)		(0·31)		
Phenol (g)	0·23	0·22	0·21	0·65	0·42	—	0·50
	(0·10)	(0·09)	0·20	(0·08)	0·40		(0·02)
			(0·09)		(0·06)		
Diphenyl (g)	1·05	1·69	1·46	5·10	5·10	1·50	10·0
	(0·29)	(0·44)	1·39	(0·40)	5·10	1·40	(0·26)
			(0·38)		(0·40)	(0·33)	
Quaterphenyl (g)	0·19	0·22	0·16	0·30	0·60	0·12	1·35
	(0·03)	(0·03)	0·16	(0·01)	0·60	0·12	(0·02)
			(0·02)		(0·025)	(0·01)	
Quinhydrone‡ (g)	—	0·38	0·36	0·33	1·20§	—	—
		(0·03)	—	(0·01)	1·20		
			(0·03)		(0·03)		
High-boiling residue (g)	2·30	2·19‖	2·09¶	6·20⊥	6·00**	3·10	17·5
			2·05		6·00	3·20	

* Reaction at 52 °C.

† Reaction at 80 °C (Gelissen and Hermans' (1925) results).

‡ This substance was isolated as follows : The high-boiling residues were further distilled to yield fractions, b.p. 150–250 °C/0·45 mm. Sublimation of these fractions (220–280 °C/17 mm) yielded yellow microneedles, which after crystallization from ethanol or benzene had m.p. 307 °C (Found (samples from two separate experiments): C, 94·0, 93·6; H, 6·0, 6·3%. Calc. for $C_{24}H_{18}.C_{24}H_{20}$: C, 93·8; H, 6·2%). The substance is far more soluble in ethanol than is quaterphenyl ; it gives a cherry-red solution in warm 36N sulphuric acid, unlike quaterphenyl which gives a brown solution ; further, it shows a bright yellow fluorescence in ultraviolet light, whereas quaterphenyl shows a blue fluorescence. It is thought to be the " quinhydrone " of quaterphenyl and dihydroquaterphenyl (cf. quinquephenyldihydroquinquephenyl, Muller and

III. DISCUSSION

Since the sum of the yields of carbon dioxide, 1st benzoic acid, and 2nd benzoic acid is very close to 2 mol in each of experiments 2–7 (Table 1) and the yields of the other reaction products are quite reproducible, a reasonably quantitative assessment of the fate of the participating entities can be made. In the following discussion, the (mean) results of experiments 6 and 7 have been chosen as representative examples. The mechanisms of formation of the various reaction products will be discussed with reference to these results, and a balanced equation will be derived for the total reaction.

(a) Mechanisms of Product Formation

(i) *Carbon Dioxide.*—At 78 °C, the yield of carbon dioxide shows a small decrease ($1 \cdot 36$–$1 \cdot 13$ mol) with increasing peroxide concentration. This effect is in agreement with previous observations (McClure, Cuthbertson, and Robertson 1942 ; Barnett and Vaughan 1947 ; Hartman, Sellers, and Turnbull 1947). It is generally accepted (Waters 1946 ; Dewar 1949) that carbon dioxide arises from thermal decomposition of benzoyloxy radicals formed by dissociation of benzoyl peroxide, that is,

$$0 \cdot 565(Ph.CO_2)_2 \rightarrow 1 \cdot 13Ph.CO_2, \quad \ldots\ldots\ldots\ldots\ldots \textbf{(1)}$$

$$1 \cdot 13Ph.CO_2 \longrightarrow 1 \cdot 13Ph. + 1 \cdot 13CO_2. \quad \ldots\ldots\ldots \textbf{(2)}$$
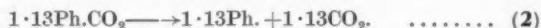
(ii) *Diphenyl and 1st Benzoic Acid.*—The mechanism of free-radical phenylation of aromatic compounds has been discussed at length in a recent symposium (Faraday Society 1953). It appears likely that reaction proceeds by addition

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Topel 1939) ; partial confirmation is afforded by its conversion into quaterphenyl (m.p. 309 °C $\varepsilon_{max.}$ at 2980 Å $= 39500$) by selenium in boiling quinoline (Gillam and Hey 1939 ; Muller and Topel 1939).

§ In these experiments, *p*-terphenyl ($0 \cdot 10$, $0 \cdot 10$ g, $0 \cdot 01$ mol) was obtained from the fractions, b.p. 150–250 °C/$0 \cdot 45$ mm. Sublimation (140–190 °C/17 mm), followed by crystallization (benzene–ethanol), yielded needles, m.p. 200 °C and mixed m.p. 202 °C.

‖ Found : C, $86 \cdot 5$; H, $6 \cdot 6\%$; mol. wt., 321.

¶ The material, dissolved in benzene, was chromatographed on alumina, and the first band (brilliant blue fluorescence in ultraviolet light) eluted and evaporated, yielded a yellow gum ($0 \cdot 36$ g). Diene-analysis, using the method of Ellis and Jones (1936), indicated 90% conjugated diene, assuming a mol. wt. of 300.

⊥ The material was dissolved in benzene and chromatographed on alumina. Elution with benzene gave four fractions, and a further three were obtained after sectioning of the column and extraction with ethanol (Soxhlet). The fractions analysed as follows :

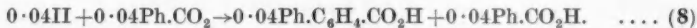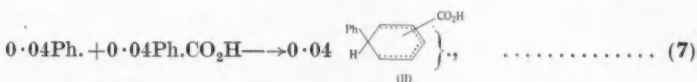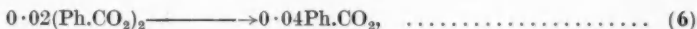| Fraction :	1	2	3	4	5	6	7
Weight (g) ..	$0 \cdot 9$	$0 \cdot 8$	$1 \cdot 1$	$0 \cdot 6$	$0 \cdot 7$	$1 \cdot 7$	$0 \cdot 4$
Carbon (%) ..	$91 \cdot 0$,	$89 \cdot 1$,	$88 \cdot 5$	$87 \cdot 6$	$85 \cdot 0$,	$84 \cdot 0$,	$77 \cdot 7$
	$90 \cdot 5$	$89 \cdot 4$			$85 \cdot 5$	$83 \cdot 7$	
Hydrogen (%) ..	$6 \cdot 2$,	$6 \cdot 3$,	$6 \cdot 7$	$6 \cdot 8$	$6 \cdot 7$,	$6 \cdot 6$,	$6 \cdot 4$
	$6 \cdot 6$	$6 \cdot 6$			$6 \cdot 7$	$6 \cdot 6$	

Fractions 1 and 2 possessed blue fluorescences in ultraviolet light ; fractions 6 and 7 were analysed for active hydrogen (Found : $0 \cdot 39$, $0 \cdot 57$, $0 \cdot 59\%$ respectively).

** Found : C, $88 \cdot 8$; H, $6 \cdot 3\%$.

of a phenyl radical to the aromatic molecule (Waters 1941), yielding a mesomeric addition product I; conversion into a substituted diphenyl is effected by reaction with a benzoyloxy radical. It has been pointed out by Szwarc (1953) that since the stationary concentration of benzoyloxy radicals is much greater than that of phenyl radicals, the most probable route of reaction will involve disproportionation between the adduct I and a benzoyloxy radical, yielding a substituted diphenyl and an equivalent amount of benzoic acid; for benzene, the reaction can be formulated as:

$$0 \cdot 205(Ph.CO_2)_2 \longrightarrow 0 \cdot 41Ph.CO_2., \quad \dots \dots \dots \quad (3)$$

$$0 \cdot 41Ph. + 0 \cdot 41PhH \longrightarrow \quad \text{(I)} \quad ., \quad \dots \dots \dots \quad (4)$$

$$0 \cdot 41I + 0 \cdot 41Ph.CO_2 \rightarrow 0 \cdot 41Ph.Ph + 0 \cdot 41Ph.CO_2H. \quad \dots \dots \quad (5)$$
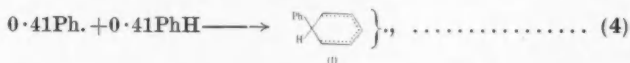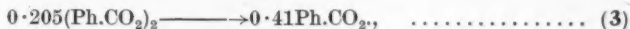
The equivalent weight of the 1st benzoic acid increases with increasing peroxide concentration; this is very probably due to phenylation of the benzoic acid formed in reaction (5). Dannley and Gregg (1954) have already shown that phenylbenzoic acids are formed when benzoyl peroxide reacts with some aromatic compounds. Increasing the peroxide concentration will, of course, lead to more extensive formation of phenylbenzoic acids. In the examples being considered, the equivalent of the 1st benzoic acid was 129, corresponding to $9 \cdot 2$ mole per cent. of phenylbenzoic acids, which are presumably formed as follows:

$$0 \cdot 02(Ph.CO_2)_2 \longrightarrow 0 \cdot 04Ph.CO_2, \quad \dots \dots \dots \dots \quad (6)$$

$$0 \cdot 04Ph. + 0 \cdot 04Ph.CO_2H \longrightarrow 0 \cdot 04 \quad \text{(II)} \quad ., \quad \dots \dots \dots \quad (7)$$

$$0 \cdot 04II + 0 \cdot 04Ph.CO_2 \rightarrow 0 \cdot 04Ph.C_6H_4.CO_2H + 0 \cdot 04Ph.CO_2H. \quad \dots \quad (8)$$

(iii) *Phenyl Benzoate.*—Phenol ($0 \cdot 06$ mol), formed by hydrolysis of phenyl benzoate, is isolated as a minor reaction product. Phenyl benzoate could be formed by the direct benzoyloxylation of benzene, that is,

$$Ph.CO_2 + PhH \longrightarrow Adduct \text{ similar to } I,$$
$$Adduct + Ph.CO_2 \rightarrow Ph.CO_2Ph + Ph.CO_2H,$$
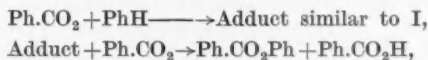
but this mechanism cannot be favoured, as the 1st benzoic acid has been virtually accounted for in reactions (5) and (8). Hey and Waters (1937) suggested that phenyl benzoate is formed by combination of free phenyl and benzoyloxy radicals; however, as the yield of phenyl benzoate does not vary appreciably with increasing peroxide concentration, Greene (1955) has suggested that it arises from an intramolecular decomposition of benzoyl peroxide, that is,

$$0 \cdot 06(Ph.CO_2)_2 \rightarrow 0 \cdot 06Ph.CO_2Ph + 0 \cdot 06CO_2. \quad \dots \dots \dots \quad (9)$$

(iv) p-*Terphenyl*.—It is assumed that this product is formed by further phenylation of diphenyl (cf. reactions (6), (7), and (8)), that is,

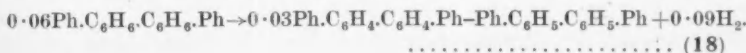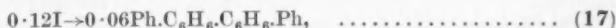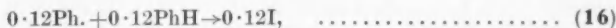$$0 \cdot 005(Ph.CO_2)_2 \longrightarrow 0 \cdot 01Ph.CO_2., \quad \dots\dots\dots\dots\dots\dots \quad (10)$$

$$0 \cdot 01Ph.Ph + 0 \cdot 01Ph. \longrightarrow 0 \cdot 01 \quad Ph\left\langle\!\!\!\begin{array}{c} H \\ Ph \end{array}\right\}., \quad \dots\dots\dots\dots\dots \quad (11)$$

(III)

$$0 \cdot 01III + 0 \cdot 01Ph.CO_2. \rightarrow 0 \cdot 01Ph.C_6H_4.Ph + 0 \cdot 01Ph.CO_2H. \quad \dots\dots \quad (12)$$
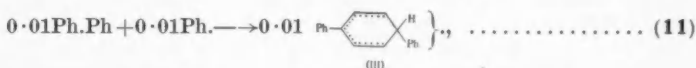
(v) *4,4′-Diphenyldiphenyl (Quaterphenyl)*.—It is considered very unlikely that quaterphenyl is formed by further phenylation of terphenyl, because quaterphenyl is formed in higher yield, while the ratio of diphenyl to terphenyl is 40 : 1. Further, Cadogan, Hey, and Williams (1954) have shown that introduction of a phenyl group into an aromatic molecule causes a roughly fourfold increase in reactivity towards phenylation ; in the presence of a large excess of unattacked solvent molecules, successive reactions should be unimportant. We suggest, therefore, that quaterphenyl is formed by dimerization of the initial adduct I between benzene and a phenyl radical, forming a tetrahydro-quaterphenyl, followed by oxidation. It is also proposed that the oxidation process is effected by means of molecular benzoyl peroxide (not benzoyloxy radicals), which becomes converted into benzoic anhydride. This suggestion is supported since the yield of 2nd benzoic acid (i.e. that liberated by hydrolysis ; $0 \cdot 31$ mol) is much greater than that of phenol ($0 \cdot 06$ mol) ; part of this excess can then be accounted for by hydrolysis of benzoic anhydride. The alternative oxidation mechanism by way of hydrogen abstraction forming benzoic acid cannot be supported, as the 1st benzoic acid has been completely accounted for (reactions (5), (8), and (12)). It is already known (Challenger and Wilson 1927 ; Horner and Jurgeleit 1955) that tertiary phosphines react with benzoyl peroxide yielding phosphine oxides and benzoic anhydride. The suggested mechanism for quaterphenyl formation is then :

$$0 \cdot 05Ph. + 0 \cdot 05PhH \rightarrow 0 \cdot 05I, \quad \dots\dots\dots\dots\dots\dots \quad (13)$$

$$0 \cdot 05I \rightarrow 0 \cdot 025Ph.C_6H_6.C_6H_6.Ph, \quad \dots\dots\dots\dots\dots\dots \quad (14)$$

$$0 \cdot 025Ph.C_6H_6.C_6H_6.Ph + 0 \cdot 05(Ph.CO_2)_2 \rightarrow$$
$$0 \cdot 025Ph.C_6H_4.C_6H_4.Ph + 0 \cdot 05(Ph.CO)_2O + 0 \cdot 05H_2O. \quad \dots \quad (15)$$
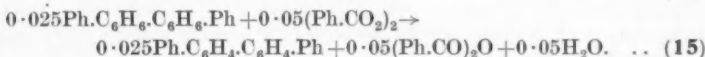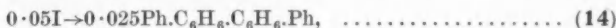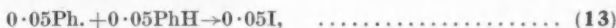
This mechanism will be supported further by evidence presented in Part II of this series (Pausacker 1957).

(vi) " *Quinhydrone*."—It is thought that this product is formed by thermal decomposition of the tetrahydroquaterphenyl formed by dimerization of the adduct I, that is,

$$0 \cdot 12Ph. + 0 \cdot 12PhH \rightarrow 0 \cdot 12I, \quad \dots\dots\dots\dots\dots\dots \quad (16)$$

$$0 \cdot 12I \rightarrow 0 \cdot 06Ph.C_6H_6.C_6H_6.Ph, \quad \dots\dots\dots\dots\dots\dots \quad (17)$$

$$0 \cdot 06Ph.C_6H_6.C_6H_6.Ph \rightarrow 0 \cdot 03Ph.C_6H_4.C_6H_4.Ph - Ph.C_6H_5.C_6H_5.Ph + 0 \cdot 09H_2.$$
$$\dots\dots\dots\dots\dots\dots\dots\dots \quad (18)$$

(vii) *High-Boiling Residue.*—Summation of the above reactions shows that although 1·13 mol phenyl radicals were formed (reaction (2)), only 0·63 mol has been used (reactions (4), (7), (11), (13), and (16)). The sum of the carboxyl-containing products (1·95 mol) indicates that 0·975 mol of benzoyl peroxide has reacted, yet only 0·905 mol has been used (reactions (1), (3), (6), (9), (15)). Further, only 0·16 mol of 2nd benzoic acid has been accounted for (reactions (9) and (15)), and 0·31 mol was found. This suggests that the 0·07 mol of benzoyl peroxide which has not been accounted for was originally incorporated in the high-boiling material as benzoate groups, which on hydrolysis yield non-phenolic hydroxyl groups and benzoic acid.

(IV)

(V)
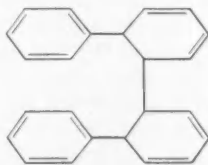
(VI)

The high-boiling residue (6·0 g) must therefore contain 0·50 mol phenyl groups derived from free phenyl radicals ($\equiv$ 3·18 g), and 0·14–0·15 mol hydroxyl groups ($\equiv$ 0·21 g). As the analytical values (see Section II) indicated 4·9 per cent. oxygen ($\equiv$ 0·29 g), a small amount of oxygen must have been absorbed, presumably during alkaline hydrolysis. Gelissen and Hermans (1925) found that their high-boiling material contained 10·1 per cent. oxygen. The difference between the weight of high-boiling material and the sum of the weights of absorbed oxygen, phenyl, and hydroxyl groups is 2·53 g, corresponding to the incorporation of 0·40 mol benzene into the material. As the molar ratios of phenyl radical and benzene in the high-boiling residue are roughly equivalent, we consider that it is a mixture of the substances formed according to reaction (17) (e.g. structures IV–VI), their polymerization products, and benzoyl-oxylated derivatives. Chromatography of the material from a similar experi-

ment (see Section II) shows that it can be resolved into a number of fractions having different oxygen contents.   Some of the material must contain conjugated double bonds, as the first fraction obtained by chromatography of the high-boiling material from Expt. 3 showed that a considerable amount of conjugated diene was present.

(b) Total Reaction Equation

Incorporating the above results, the total reaction can be written as follows :

$$1 \cdot 00(Ph.CO_2)_2 + 0 \cdot 98PhH \rightarrow 1 \cdot 19CO_2 + 0 \cdot 42Ph.CO_2H + 0 \cdot 04Ph.C_6H_4.CO_2H$$
$$+ 0 \cdot 40Ph.Ph + 0 \cdot 06Ph.CO_2Ph + 0 \cdot 01Ph.C_6H_4.Ph + 0 \cdot 025Ph.C_6H_4.C_6H_4.Ph$$
$$+ 0 \cdot 05(Ph.CO)_2O + 0 \cdot 05H_2O + 0 \cdot 06Ph.C_6H_6.C_6H_6Ph + (0 \cdot 50Ph, 0 \cdot 40PhH,$$
$$0 \cdot 15Ph.CO_2).$$
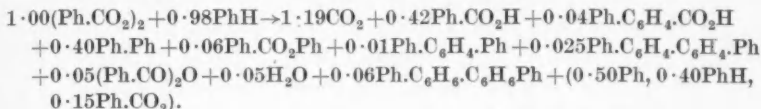
The equation accounts for the stoichiometry of the reaction very satisfactorily. Similar equations may be written to express the total reactions at other concentrations.   It should be noted that approximately 1 mole of benzoyl peroxide reacts with 1 mole of benzene.

(c) The Suitability of Benzoyl Peroxide as a Reagent for Evaluating Reactivities towards Free Radicals

The complex nature of this reaction indicates strongly that benzoyl peroxide may not be a suitable reagent for determining the relative reactivities towards free radicals.   However, Hey, Stirling, and Williams (1956) have carried out an extensive programme of experiments in which they have measured the yields of substituted diphenyls formed in the reaction of benzoyl peroxide with a large excess of an equimolar mixture of two aromatic compounds ; this is the " competitive " method, developed by Ingold and Shaw (1927), which has been employed successfully in evaluating the relative reactivities of aromatic compounds towards electrophilic reagents.   From their results Hey and his co-workers have calculated the relative reactivities of the *ortho*-, *meta*-, and *para*-positions of aromatic compounds (expressed in terms of " partial rate factors ") towards free-radical substitution.   We believe that this method, which involves selection of one reaction path (diphenyl formation) for evaluation of total reactivity towards phenyl radicals, can be only very approximate when benzene is one of the components of a mixture used in a " competitive " experiment. Augood, Hey, and Williams (1952) have carried out competitive experiments with benzene and equimolar amounts of chlorobenzene, nitrobenzene, and pyridine respectively.   Rondestvedt and Blanchard (1956) have also criticized the application of the " competitive " method in studies of free-radical reactivities and state that " although the competitive method is the best available technique for comparing reactivities, great reliance must not be placed on the numerical values of the total and partial rate factors, except when comparing compounds where the side reactions are unimportant ".

In the example under discussion only 80 per cent. of the benzoyl peroxide undergoes reaction with benzene.   The remainder (1) effects either phenylation of benzoic acid (reactions (**6**) to (**8**)) or of diphenyl (reactions (**10**) to (**12**)); (2) undergoes intramolecular decomposition (reaction (**9**)) ; (3) acts as an oxidant

(reaction (**15**)) ; or (4) becomes incorporated in the high-boiling residue. Further, only 40 per cent. of the adduct I formed between benzene and a phenyl radical (reactions (**4**), (**13**), (**16**) ; discussion of high-boiling residue) reacts with benzoyloxy radicals to form diphenyl (reaction (**5**)). The remainder is either converted into quaterphenyl (reactions (**14**) to (**15**)), "quinhydrone" (reactions (**17**) to (**18**)), or high-boiling residue.

IV. ACKNOWLEDGMENTS

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REACTIONS OF AROYL PEROXIDES

II. SUBSTITUTED BENZOYL PEROXIDES WITH BENZENE

By K. H. PAUSACKER*

Summary

Six symmetrically disubstituted benzoyl peroxides have been reacted with benzene and the nature of the products has been determined. The reactions of the *m*-methoxy-, *p*-methoxy-, *p*-chloro-, and *p*-methylbenzoyl peroxides are very similar to the reaction of benzoyl peroxide itself (Part I of this series (Lynch and Pausacker 1957)), but it has been found that *m*-nitro- and *p*-nitrobenzoyl peroxide give very much higher yields of the corresponding diphenyl. This is explained by assuming that the latter two compounds are more susceptible to induced decomposition.

I. INTRODUCTION

Although the kinetics of the spontaneous thermal decomposition of substituted benzoyl peroxides has been investigated in detail (Swain, Stockmayer, and Clarke 1950 ; Blomquist and Buselli 1951), little information is available concerning the nature of the products formed when substituted benzoyl peroxides react with aromatic compounds. Hey and Walker (1948) have investigated the reactions of benzene with *o*-, *m*-, and *p*-nitro-, *p*-methoxy-, and *o*-methoxycarbonylbenzoyl peroxide with benzene at 78 °C and have found that 0·58, 0·78, 0·52, 0·23, and 0·50 mol† of the corresponding diphenyls are formed.

In the present work, the products formed when *m*- and *p*-nitro, *m*- and *p*-methoxy-, *p*-chloro-, and *p*-methylbenzoyl peroxide react with benzene at 78 °C have been isolated.

II. EXPERIMENTAL

Analyses were made by the C.S.I.R.O. Microanalytical Laboratory.

(a) Materials

The disubstituted benzoyl peroxides were prepared by standard methods (Swain, Stockmayer, and Clarke 1950) and were found to have a minimum purity of 98 per cent. by iodometric analysis. Thiophen-free benzene, dried over sodium, was used as solvent.

(b) Method

The reactions were carried out, and worked up, by a procedure which was identical with that already described in Part I (Lynch and Pausacker 1957). The results are given in Table 1. The values in parentheses represent the average yields expressed as mols. All experiments were performed at 78 °C.

* Chemistry Department, University of Melbourne.
† Per mole of substituted benzoyl peroxide.

D

TABLE 1

PRODUCTS FORMED FROM THE REACTION OF AROYL PEROXIDES WITH BENZENE

Substituent:	m-NO$_2$	p-NO$_2$	m-OMe	p-OMe	p-Cl	p-Me	H (Lynch and Pausacker 1957)
Weight (g)	8·4	8·4	8·6	8·6	8·9	7·8	6
Volume PhH (ml)	300	300	300	300	300	300	200
Molarity	0·084	0·084	0·095	0·095	0·094	0·096	0·124
Time of heating (hr)	19, 26	25, 26	18, 9	9, 10	26, 24	19, 15	22, 28
Volume CO$_2$ (ml)	500, 510 (0·89)	520, 534 (0·93)	693, 636 (1·06)	—[8]	768, 705 (1·17)	740, 694 (1·13)	(1·36)
Weight of 1st acid (g)	—, 4·0 (0·95)	4·1, 4·2 (0·98)	2·50, 2·60 (0·59)	2·40, 2·50 (0·57)	2·00, 1·60 (0·40)	1·50, 1·50 (0·38)	(0·42)
M.p. of 1st acid (°C)	139	242	94	180	242	178	—
Weight of 2nd acid (g)	0·40,[11] 0·20[11] (0·07)	0·25,[11] (0·06)	1·50, 1·70 (0·38)	1·90, 1·65 (0·42)	1·90, 2·40 (0·48)	1·50, 1·65 (0·40)	(0·25)
M.p. of 2nd acid (°C)		242	90	181	242	179	—
Phenol (g)	0·25, 0·20 (0·06)	0·15,[1] (0·04)	0·50,[3] 0·40[4] (0·13)	0·35, 0·30[9] (0·10)	0·30[11] 0·30[13] (0·10)	0·30, 0·55 (0·14)	(0·09)
Diphenyl (g)	4·4, 4·6 (0·90)	4·7, 4·8 (0·94)	2·30, 2·25 (0·44)	1·80, 1·70 (0·34)	2·50, 2·35 (0·47)	2·00, 2·55 (0·47)	(0·38)
B.p. diphenyl (°C/mm)	145/0·4	150/0·3	125/0·7	113/0·3	119/0·4	97/0·3	—
Quaterphenyl (g)			0·30,[13] 0·30 (0·03)		0·35,[13] 0·25 (0·03)	0·20,[16] 0·15 (0·03)	(0·02)
Residue (g)	0·5, 0·5	0·2 , 0·5[12]	2·40,[14] 2·05[7]	2·60, 3·50[10]	2·70,[14] 2·80[15]	2·70, 2·35	2·07

[1] The residue could not be hydrolysed with alkali as azoxy-compounds were formed. Alcoholic hydrochloric acid was used.
[2] The residue was chromatographed over alumina in benzene solution. The product from the first band was crystallized from ethanol giving a substance, m.p. 163 °C (Found: C, 67·5; H, 4·1; N, 8·9%. Calc. for C$_{16}$H$_{10}$O$_2$N$_2$: C, 67·5; H, 3·7; N, 8·8%). It is probably a mixture of 4-nitroterphenyls as the m.p. does not correspond to any of the known 4-nitroterphenyls.
[3] Found: OMe, 17·5%. [4] Found: OMe, 17·3%.
[5] Pale yellow needles, crystallizing from xylene, m.p. 221 °C (Found: C, 85·1; H, 6·4; OMe, 15·7%. Calc. for C$_{20}$H$_{18}$O$_3$: C, 85·2; H, 6·0; 2OMe, 16·9%).
[6] Found: OMe, 14·6%. [7] Found: OMe, 14·8%. [8] Not measured. [9] Found: OMe, 17·5%. [10] Found: Cl, 16·1%.
[11] Found: Cl, 18·1%.
[12] Yellow plates, crystallizing from xylene, m.p. 330 °C (Found: C, 77·3; H, 4·5; Cl, 18·4%. Calc. for C$_{24}$H$_{14}$Cl$_2$: C, 76·8; H, 4·3; Cl, 18·9%).
[13] Found: Cl, 17·6%.
[14] Found: Cl, 16·7%.
[15] Found: Cl, 17·6%.
[16] Yellow needles, crystallising from xylene, m.p. 350 °C (Found: C, 93·0; H, 6·8%. Calc. for C$_{24}$H$_{20}$: C, 93·4; H, 6·6%).

III. DISCUSSION

When the yields of the products formed in the reactions of m-methoxy-, p-methoxy-, p-chloro-, and p-methylbenzoyl peroxide are compared with those formed in the reaction of benzoyl peroxide with benzene, it is seen there is a remarkable similarity. Apparently the introduction of these substituents does not seriously alter the course of the reaction.

Balanced equations may be written for all these reactions (cf. the reaction of benzoyl peroxide, Part I of this series (Lynch and Pausacker 1957), for details) and they are similar to those already quoted in Part I. These equations will not be written in detail but the more interesting results are given in Table 2.

TABLE 2

SUMMATION OF PRODUCTS

Substituent	m-OMe	p-OMe*	p-Cl	p-Me
Sum of 1st acid, 2nd acid, and carbon dioxide	1·93, 2·01	—	2·08, 2·01	1·93, 1·88
Substituent (%) accounted for	96, 97	—, 96	96, 95	—
Moles PhH reacting ..	1·08, 0·96	0·82, 1·06	1·07, 1·04	0·89, 1·09
High-boiling residue				
Moles Ar.	0·40, 0·35	0·46, 0·56	0·47, 0·47	0·58, 0·42
Moles PhH	0·51, 0·43	0·37, 0·63	0·47, 0·52	0·40, 0·40
Moles ArCO$_2$.	0·11, 0·23	0·38, 0·28	0·11, 0·23	0·30, 0·23

* A yield of 0·03 mole quaterphenyl is assumed.

It can be seen that these reactions are accounted for reasonably quantitatively as the sum of " first acid ", " second acid ", and carbon dioxide is close to 2·00. In those cases where estimable functional groups are present, at least 95 per cent. of the aroyl peroxide has been accounted for.

The difference in weights of the products and reactants must be equal to the weight of benzene that reacts with the aroyl peroxide, and, when this is expressed in molar quantities, it is seen (Table 2) that approximately 1 mole of benzene reacts per mole of aroyl peroxide. This is in agreement with the results obtained in Part I and is also noted when benzoyl peroxide reacts with a number of other aromatic compounds (unpublished data).

In addition, it is seen (Table 2) that the ratio of aryl radical to benzene is approximately unity in the high-boiling residue, thus verifying the suggestions made in Part I concerning the composition of this material. It is assumed that an intermediate I is formed by the initial reaction of an aryl radical with benzene and then I undergoes further reaction forming either a diphenyl or high-boiling material, quaterphenyl, etc.

$$PhH + Ar. \rightarrow \quad \overset{Ar}{\underset{H}{\bigcirc}} \left. \begin{array}{c} \xrightarrow{Ph.CO_2} Ph.Ar + Ph.CO_2H \\ \\ \searrow \text{High-boiling material,} \\ \text{quaterphenyls, etc.} \end{array} \right\} \quad .. (1)$$
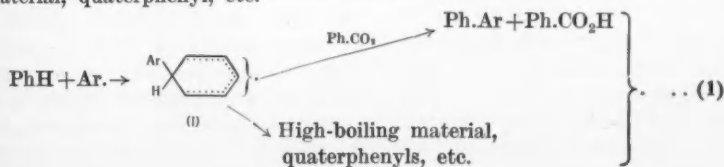
In every case (Table 1) the weight of diphenyl formed is slightly less than that of the high-boiling material. Further support is given to the suggestion made in Part I that these reactions are not suitable for the quantitative determination of the reactivity of aromatic molecules towards free-radical attack, simply by comparing the relative yields of diphenyls formed in competitive reactions. It is of interest to note that the quaterphenyls formed in these reactions are of the type $Ar.C_6H_4.C_6H_4.Ar$. This would indicate that they are formed by the reactions:

$$2Ar.+2C_6H_6 \rightarrow 2Ar.C_6H_6. \rightarrow Ar.C_6H_6.C_6H_6.Ar \underset{-4H}{\longrightarrow} Ar.C_6H_4.C_6H_4.Ar$$
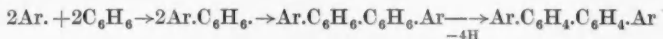
rather than by successive arylation of the diphenyl initially formed in the reaction. In this latter case the formula would be expected to be

$$Ar.Ar(-H).Ar(-H).Ph.$$
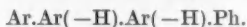
No attempt has been made to determine the position of the substituents in these quaterphenyls which could be a mixture of more than a single substance.

A marked difference is noted in the composition of the products when m- or p-nitrobenzoyl peroxide reacts with benzene. It is seen (Table 1) that at least 90 per cent. of the total reaction may proceed as follows:

$$(Ar.CO_2)_2+Ph.H \rightarrow Ph.Ar+Ar.CO_2H+CO_2.$$
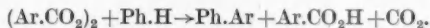
As the side-reactions involved are not appreciable, it is possible that these two aroyl peroxides could be more profitably used in a study of the relative reactivities of aromatic molecules with free radicals. It is intended to investigate the reaction of these two peroxides with other aromatic compounds in order to see if this simple reaction is always predominant.

From kinetic studies it is known (Cass 1946; Nozaki and Bartlett 1946, 1947; Barnett and Vaughan 1947) that the reaction of benzoyl peroxide with aromatic compounds involves either a first-order, spontaneous thermal decomposition into benzoyloxy radicals, that is,

$$(Ph.CO_2)_2 \rightarrow 2Ph.CO_2,$$
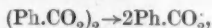
and/or an induced decomposition, for example,

$$(Ph.CO_2)_2 \rightarrow 2Ph.CO_2,$$
$$Ph.CO_2 \rightarrow Ph.+CO_2,$$
$$Ph\cdot+ArH \rightarrow Ph.ArH\cdot,$$
$$Ph.ArH\cdot+(PhCO_2)_2 \rightarrow Ph.Ar+Ph.CO_2H+Ph.CO_2.$$
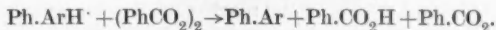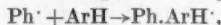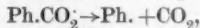
The order of the induced decomposition is dependent on the chain-termination mechanism. Thus Barnett and Vaughan (1947) have established that the reaction of benzoyl peroxide with benzene (80 °C) is first-order only at infinite dilution. At all finite concentrations, the thermal decomposition is accompanied by a reaction of higher order. Furthermore Nozaki and Bartlett (1946, 1947) have shown that at least part of the reaction of benzoyl peroxide with benzene involves induced decomposition since it responds to inhibitors (e.g. hydro-

quinone, oxygen) and to free-radical initiators (e.g. tetraphenylhydrazine, hexaphenylethane).

It has been shown (Swain, Stockmayer, and Clarke 1950) that electron-attracting substituents in the benzoyl peroxide make it much more susceptible to induced decomposition, because these substituents contribute additional stabilizing resonance forms in the postulated transition state. The equivalent yields of diphenyls, free-aromatic acids, and carbon dioxide that are found in the reactions of m- and p-nitrobenzoyl peroxides with benzene are therefore attributed to the marked susceptibility of these peroxides towards induced decomposition which becomes the main path of reaction in these systems. It is considered that the reactions of the other aroyl peroxides with benzene involve both spontaneous thermal decomposition and induced decomposition.

A distinct correlation exists when the average times at which half the total amount of carbon dioxide is evolved is compared with the rate constants (Blomquist and Buselli 1951) of the thermal decompositions of these aroyl peroxides (Table 3).

<div align="center">TABLE 3</div>

<div align="center">RATES OF REACTION OF AROYL PEROXIDES WITH BENZENE</div>

Substituent	m-NO$_2$	p-Cl	—	p-NO$_2$	p-Me	m-OMe	p-OMe
$k \times 10f$ (80 °C, min^{-1}) ..	2·28	2·30	2·59	2·60	3·55	3·85	9·35
$t\frac{1}{2}$ (hr)	5·6	4·7	6·0	7·0	4·4	3·1	2·4

It would thus appear that aroyl peroxides react with benzene according to two distinct (and probably simultaneous) mechanisms. It may be noted that the relative reactivities of benzene, chlorobenzene, and nitrobenzene are $1 : 1 \cdot 45 : 4 \cdot 0$ when benzoyl peroxide is used and $1 : 1 \cdot 03 : 1 \cdot 53$ when p-chlorobenzoyl peroxide is the arylating reagent (Cadogan, Hey, and Williams 1955). This difference may be due to the different contributions of spontaneous thermal decomposition and induced decomposition in the systems studied but it is also possible that the differences could be explained by the different (electrophilic) reactivities of the phenyl and p-chlorophenyl radicals. Furthermore, it has been shown (Cadogan, Hey, and Williams 1954) that benzoyl peroxide reacts with an equimolar mixture of nitrobenzene and p-ditert.-butylbenzene yielding $0 \cdot 53$ mol of total diphenyls, while $0 \cdot 88$ mol of total diphenyls are obtained from a similar reaction with nitrobenzene and p-dichlorobenzene (Augood, Hey, and Williams 1953). In these two reactions (which have been used in the evaluation of relative reactivities) the amounts of benzoyl peroxide used in phenylation are in the ratio of $1 : 1 \cdot 66$. It is apparent that a widely variable proportion of side reactions occurs when benzoyl peroxide reacts with aromatic compounds and that the measurement of the diphenyls formed may only give a qualitative measure of the relative reactivities of aromatic compounds. However, it is possible that m- and p-nitrobenzoyl peroxides may be usefully employed in this regard due to the high yield of diphenyls from these reactions.

K. H. PAUSACKER

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THE SEPARATION OF SUGARS ON CARBON COLUMNS BY GRADIENT ELUTION

By M. A. JERMYN*

Summary

The influence of various factors on the elution of sugars from charcoal columns by concentration gradients of organic substances dissolved in water can be readily explained, to the first approximation at least, by considering the sugar and the desorbing agent as competitors for the same active surface. It is possible to predict the behaviour of various systems on this hypothesis and to show how various types of separation can best be achieved. It is possible to separate the series of cello-oligoglucosides from acid-hydrolysed cellulose with the minimum of manipulation by using suitable conditions suggested by this study.

I. INTRODUCTION

The technique of Whistler and Durso (1950) for the separation of oligosaccharides on carbon columns by elution with aqueous ethanol has recently been extended (Lindberg and Wickberg 1953 ; Barker, Bourne, and Theander 1955) by the use of gradient elution. In this technique the concentration of an eluant dissolved in a flowing carrier liquid is varied continuously as the eluting solution flows down the column. The nature of the concentration gradient of eluant is determined by the device in which eluant and carrier are mixed. Adsorbed substances are eluted from the column in the order of their ease of desorption by the eluant, and substances of very different affinities for the column material can be removed in a single operation. The steadily increasing eluant concentration also tends to eliminate the normal " tailing " in the desorption of the last fractions of an adsorbed substance when a fixed concentration of eluant is used.

However, there is as yet no general theory by which to predict the behaviour of a given system (Drake 1955). In these laboratories it was desired to separate a series of oligosaccharides derived from partially hydrolysed cellulose, and preliminary experiments had shown that gradient elution from charcoal columns readily separated monosaccharides from disaccharides and trisaccharides as groups as well as partially separating a number of disaccharides from each other (cf. Tu and Ward 1955).

It was possible to alter most of the variables involved in column operation and to compare the effects with those predicted by currently accepted theory. The effects of temperature (Tu and Ward 1955) and complexing agents (Barker, Bourne, and Theander 1955) which have been thoroughly investigated by other workers have not been included in this study.

* Biochemistry Unit, Wool Textile Research Laboratories, C.S.I.R.O., Melbourne.

II. Materials and Methods

The carbon used throughout was " Darco G–60 " (Darco Corp., New York), as used by Whistler and Durso (1950) in their original studies. It was mixed in equal proportions with Whatman's " Standard Chromatographic Cellulose Powder " and the mixture extracted once with boiling 50 per cent. ethanol and twice with boiling water. The extracted material was dried for 24 hr at 100 °C before use. The powder was made into a slurry with water, poured into a chromatographic tube at the end of which was a sintered glass disk and a pad of pure cellulose powder, and allowed to settle under gravity. Cellulose powder was found to give faster flow rates than " Celite " as originally recommended by Whistler and Durso (1950), and no perceptible amounts of carbohydrate were removed from it in blank experiments.

Tests showed that its adsorptive capacity for the substances used in the experiments must be several orders of magnitude less than that of the carbon, and that it could be regarded as an inert background except for the higher cellulose oligosaccharides.

The 15 g column (small column) was enclosed by a jacket filled with water circulating from a thermostat bath maintained at $28 \pm 0 \cdot 1$ °C. The mixing chamber was a single vessel sealed except for inlet and outlet tubes, the mixing being brought about by a magnetic stirrer. This arrangement gives an exponential gradient (Drake 1955). The gradient could be steepened to any desired degree by using an adjustable by-pass at the head of the column. Where the effluent concentration is still very much below that of the solution supplied to the mixing chamber the departure of the exponential curve from a straight line may be neglected, and many of the calculations in the present paper make use of this approximation. The total head of liquid above the surface of the packing was about 40 cm. The larger-scale experiments (100 g packing) were carried out in unjacketed columns operating under about 300 cm total head. The mixing chamber was fitted with a motor-driven stirrer passing through a watertight seal.

For the adsorption experiments, appropriate amounts of carbon and solution were shaken together in stoppered test tubes by a wrist-action shaker in a constant-temperature room at 28 ± 1 °C. Equilibrium was attained after a few minutes, but a shaking period of 30 min was allowed after which the contents of the tubes were filtered and the filtrates analysed.

Analyses of eluate fractions were carried out by techniques already described: reducing sugars by the Nelson (1944) technique ; total carbohydrate by the anthrone method (Jermyn 1955) ; ketoses in the presence of aldoses by the cold anthrone method (Jermyn 1956) ; and alcohols by a modification of the A.O.A.C. (1940) method for glycerol.

III. Results and Discussion

(a) Mechanism of Separation

(i) *Desorbing Substances.*—A standard experiment was devised in which a variety of water-miscible organic liquids were tested for their ability to desorb 20 mg of cellobiose from 1 g of " Darco " charcoal (10 ml total volume at

28 °C). The desorbents were tested at concentrations up to 10 per cent. (v/v). The following classes were found to act as desorbing agents : straight-chain aliphatic alcohols (in this and other homologous series the effectiveness increased as the series was ascended), straight-chain aliphatic acids, aliphatic and cyclic secondary and tertiary amines. A variety of other reagents (acetone, lactic acid, ethyl lactate, ethylene glycol mono-ethers, diethyl carbitol) were also effective. Aliphatic primary amines and simple esters were ineffective as were also such polyfunctional compounds as glycerol. Bifunctional compounds were less effective than monofunctional compounds of the same ring size or chain length. An amino function reduced the effectiveness more than a hydroxyl function. Thus ethanol> ethylene glycol> ethanolamine> ethylenediamine and piperidine> dioxan> morpholine are both series of decreasing effectiveness, with ethylenediamine and morpholine actually ineffective. Branching of the carbon chain in alcohols reduced the effectiveness ; thus *iso*propanol was less effective than *n*-propanol and the series butanol> *iso*butanol> *sec.*-butanol> *tert.*-butanol declined into complete ineffectiveness with *tert.*-butanol.

Thus not only were a wide variety of compounds effective desorbents but steric effects were obviously involved in determining their effectiveness. The effect of the molecular shape of amines on their comparative behaviour as competitors for an active surface is remarkably similar both as measured by their desorbing power in the present system and as inhibitors of the attack of hydrogen ions on metals (Hurst and Jermyn 1950).

(ii) *Effect of Desorbent on Relative Ease of Desorption of Sugars.*—Experiments with the ethanol desorption of disaccharides from charcoal columns showed that the order of ease of desorption was sucrose> lactose> cellobiose. A wide variety of desorbing agents were tried to see whether the above order could be altered or whether at least a better separation of sucrose and lactose could be obtained. Neither of these objects was achieved, the order of the sugars and their spacing remaining unaltered for the range of desorbents listed in Table 1, except the cyclic bases pyridine and piperidine which somewhat altered the spacing. However, organic bases are impractical for a number of reasons as eluting agents for reducing sugars. The mechanism of desorption appears to be identical for most of the agents listed. To illustrate this point more clearly Figure 1 sets out large portions of the desorption curves for three rather inefficient desorbents.

(iii) *Mechanism of Adsorption on Charcoal.*—To check the hypothesis that the eluting agents act by competing with the oligosaccharides for the same active sites on the carbon, the adsorption of various substances from aqueous solution on the charcoal was studied with the results shown in Figure 2. It will be seen that the adsorption of both sugars and desorbing agents fits the equation $W = Ac^b$ (the Freundlich isotherm). The constants A and b have similar values for both sugars and simple aliphatic compounds so that the amounts adsorbed (on a molar basis) are of the same order of magnitude at similar molarities. The order of the affinities of the charcoal for the sugar (lactose> cellobiose> sucrose> raffinose) is not the same as that of the tenacity with which they are held once adsorbed (raffinose> cellobiose> lactose> sucrose).

TABLE 1

ABILITY OF CERTAIN ORGANIC SUBSTANCES IN AQUEOUS SOLUTION (10 ML) TO DESORB 20 MG OF
DISACCHARIDES FROM 1 G OF " DARCO G–60 " CHARCOAL AT 28 °C

Desorbing Agent	Concentration (M) required to Achieve 50% Desorption		
	Sucrose	Lactose	Cellobiose
Acetone	0·38	0·53	0·74
n-Butanol	0·15	0·17	0·20
n-Butyric acid	0·13	0·14	0·17
Diethylamine	0·15	0·16	0·18
Diethylene glycol diethyl ether ..	0·046	0·053	0·067
Dioxan	0·29	0·35	0·46
Ethanol	1·4	1·6	2·0
Ethyl lactate	0·12	0·15	0·19
Ethylene glycol monomethyl ether ..	0·67	0·75	0·87
Ethylene glycol monobutyl ether ..	0·076	0·084	0·095
Ethylene glycol	0·73	0·90	1·25
Formic acid	2·4	2·6	2·9
Lactic acid	0·20	0·25	0·34
Methanol	4·8	5·1	6·3
Piperidine	0·21	0·14	0·21
Pyridine	0·14	0·11	0·14

The difference between the disaccharides and raffinose can most readily be
explained by supposing that fewer of the pores in the adsorbent will allow the
larger molecule to pass through them and that a molecular sieve effect (Lathe
and Ruthven 1956) is superimposed on the adsorption.

(iv) *Competition between Sugars and between Sugars and Desorbent.*—Since
both sugars and desorbents appear to be adsorbed at the same centres by the
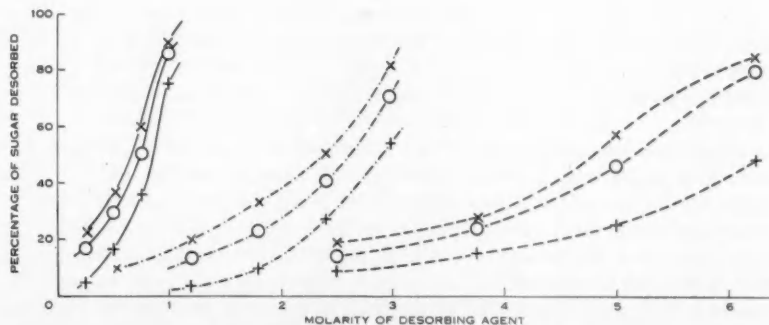
Fig. 1.—Desorption of 20 mg of three disaccharides from 1 g of " Darco G–60 " charcoal
by 10 ml of an aqueous solution of various desorbing agents at 28 °C.

    × Sucrose.   ○ Lactose.   + Cellobiose.   — Ethylene glycol monomethyl ether.
                —·— Formic acid.   — — Methanol.
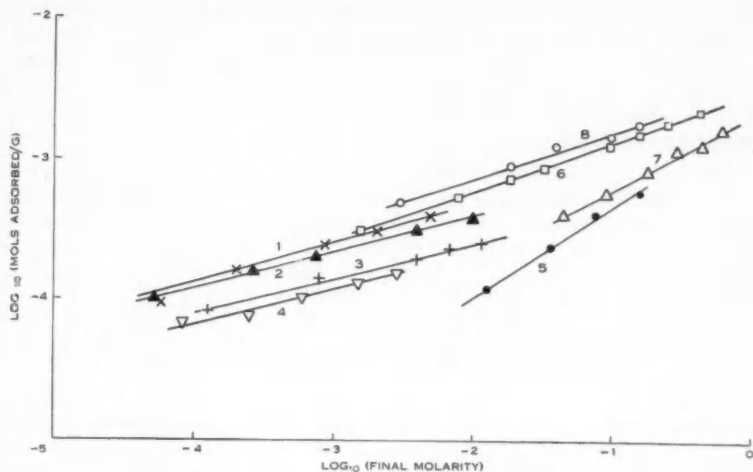
Fig. 2.—Adsorption of various substances from an aqueous solution on " Darco G–60 " charcoal at 28 °C.

1, Lactose. 2, Cellobiose. 3, Sucrose. 4, Raffinose. 5, Ethanol. 6, n-Butanol.
7, Formic acid. 8, n-Butyric acid.
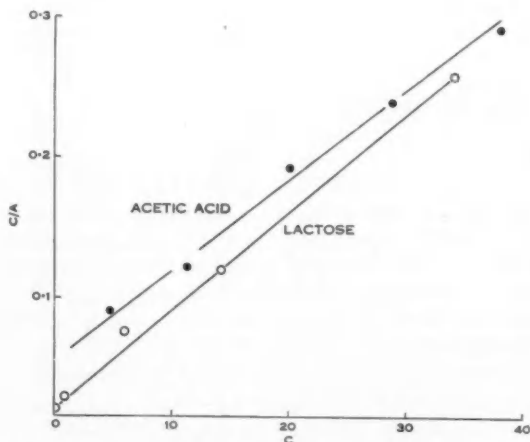
Fig. 3.—Adsorption of lactose and acetic acid on " Darco G–60 " charcoal from aqueous solution at 28 °C.

C, concentration. A, amount absorbed. Lactose units shown in mg/10 ml and mg/g charcoal; acetic acid units in m mole/100 ml and m mole/10 g charcoal.

same mechanism, it should be possible to demonstrate direct competition between two sugars or between sugar and desorbent for a limited amount of charcoal.

In Table 2 are presented the results of an experiment in which competitive adsorption of acetic acid and lactose was studied. Organic acids were used in this and similar studies because they could be readily determined by titration without interference from the sugars.

Although the adsorption of various substances has been represented above by Freundlich isotherms for convenience in condensing a wide range of data into a single figure, it is well known that these have no fundamental theoretical

TABLE 2

ADSORPTION FROM SOLUTIONS (10 ML) CONTAINING BOTH LACTOSE AND ACETIC ACID ON 1 G OF " DARCO G–60 ' CHARCOAL AT 28 °C

Solution Containing	Micromoles		Micromoles		Micromoles		Micromoles		Total Lactose Present (micromoles)
	In Solution	Ad-sorbed	In Solution	Ad-sorbed	In Solution	Ad-sorbed	In Solution	Ad-sorbed	
Lactose ..	131	163	118	175	78	216	42	251	294
Acetic acid ..	3140	895	1800	721	598	211	0	0	
Lactose ..	49	98	31	116	14	132	5·5	141	147
Acetic acid	3100	933	1710	816	543	467	0	0	
Lactose ..	20	54	12	61	3·5	70	0·6	73	74
Acetic acid ..	3030	1000	1680	845	510	500	0	0	
Lactose ..	0	0	0	0	0	0	0	0	0
Acetic acid ..	2880	1150	1565	958	478	532	0	0	
Total acetic acid (micromoles)	4032		2523		1010		0		

significance. In fact, the observations for sugars and acids can be fitted to a Langmuir isotherm and Figure 3 shows that a plot of C/A against C is a straight line for both acetic acid and lactose as required by the Langmuir equation. It is therefore reasonable to follow Langmuir's (1916) argument with the additional assumption that water is so readily displaced from the active surface by the substances studied that it need not be considered as a competitor. Then for the competitive adsorption of two substances A and B from solution on an adsorbent we have

$$n_A = K_A C_A / (1 + K'_A C_A + K'_B C_B),$$
$$n_B = K_B C_B / (1 + K'_A C_A + K'_B C_B),$$

where C_A, C_B are the concentrations of A and B at equilibrium; n_A, n_B the number of moles of A and B adsorbed; and K_A, K_B, K'_A, K'_B are constants. If either A or B is absent these equations reduce to the normal Langmuir isotherm.

Combining the two equations above gives us

$$n_A/n_B = K_A C_A / K_B C_B,$$

or

$$n_A C_B / n_B C_A = P \quad \text{(a constant)}.$$

The last equation gives a test for the existence of purely competitive adsorption for cases where the Langmuir assumptions can be expected to hold. The result of applying this test to the data of Table 2 is shown in Table 3. The same pattern was found for all possible combinations of the three sugars (lactose, sucrose, and cellobiose) and two desorbents (acetic and n-butyric acids) studied, namely, near constancy of $n_A C_B / n_B C_A$ at high concentrations of sugar and

TABLE 3

VALUES OF THE QUANTITY $n_{\text{Lac}} C_{\text{Ac}} / n_{\text{Ac}} C_{\text{Lac}}$ CALCULATED FROM TABLE 2

Total Lactose (micromoles)				
294	4·35	3·69	4·03	
147	6·65	7·98	10·75	
74	8·33	16·15	20·3	
	4032	2523	1010	Total acetic acid (micromoles)

desorbent and a trend towards decreasing efficiency of desorption at low concentrations. It appears that the first portion of the sugar bound is more strongly held than the rest.

Tables 4 and 5 show the results when the two sugars lactose and sucrose were allowed to compete for a limited amount of charcoal, either alone or in the presence of acetic acid. It appears that the relation between sucrose and lactose is almost directly one of simple competition. The case of three adsorbed substances, however, cannot be treated quantitatively since application of the Langmuir reasoning gives a complicated expression with a large number of constants, but the results of Table 5 suggest that here too simple competition is involved. Parallel results showing the differential desorption of sucrose and lactose were obtained with n-butyric acid, n-butanol, and ethanol.

(v) *Runs with Desorbent Only.*—From the experiments reported above it appeared that it would be impossible to obtain qualitatively different results in the operation of charcoal columns by using different desorbents, but that it would be possible to obtain qualitative differences in the separation of various sugars by varying the desorbent and the slope of its concentration gradient. Since these desorbents are themselves adsorbed on the column it is apparent that they will tend to emerge from the column not as a smooth gradient rising

TABLE 4

ADSORPTION OF MIXTURES OF SUCROSE AND LACTOSE FROM AQUEOUS SOLUTION (10 ML) ON 1 G OF "DARCO G-60" CHARCOAL AT 28 °C

Total Sugars (mg)				Sugars in Solution (mg)				Sugars Adsorbed (mg)				$\dfrac{n_{Lac}\,C_{Suc}}{n_{Suc}\,C_{Lac}}$
Sucrose	Lactose	Total	Sucrose (%)	Sucrose	Lactose	Total	Sucrose (%)	Sucrose	Lactose	Total	Sucrose (%)	
140·0	—	140·0	100·0	51·9	—	51·9	100·0	88·1	—	88·1	100·0	
112·0	28·6	140·6	79·7	46·2	5·3	51·5	89·8	63·8	23·3	86·9	73·5	3·2
84·0	85·8	141·8	39·5	26·1	21·4	47·5	54·9	29·9	66·4	94·3	31·7	3·3
28·0	114·4	142·4	19·7	14·1	32·3	46·4	30·4	13·9	82·1	96·0	14·5	2·5
—	143·0	143·0	0·0	—	40·0	40·0	0·6	—	103·0	103·0	0·0	

from their first appearance but as an abrupt " front ". Figure 4 shows the appearance of the fronts for n-butyric acid (strongly adsorbed) and formic acid (weakly adsorbed).

TABLE 5

ADSORPTION OF MIXTURES OF SUCROSE, LACTOSE, AND ACETIC ACID FROM 10 ML OF AQUEOUS SOLUTION ON 1 G " DARCO G–60 " CHARCOAL AT 28 °C

Total Absorbent (micromoles)			In Solution (micromoles)			Adsorbed (micromoles)		
Acetic Acid	Sucrose	Lactose	Acetic Acid	Sucrose	Lactose	Acetic Acid	Sucrose	Lactose
—	147	147	—	38	21	—	109	126
1005	147	147	607	60	37	398	87	110
2010	147	147	1428	83	53	582	64	94
3015	147	147	2205	104	60	810	43	87
4020	147	147	3007	112	67	1013	35	80

(vi) *Effect of Different Desorbents on the Same Desorbed Substance.*—Figure 5 shows that powerful desorbents such as n-butyric acid which give a sharp front carry such a relatively readily desorbed substance as lactose actually in the
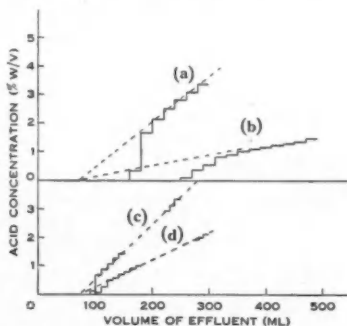
Fig. 4.—" Fronting " when a continuous gradient of a desorbent in solution was run through a small column at 28 °C.

Concentration (w/v) of solution supplied to the mixing chamber (a) 20 per cent. n-butyric acid ; (b) 5 per cent. n-butyric acid ; (c) 24 per cent. formic acid ; (d) 10 per cent. formic acid. The dotted lines give the calculated form of the gradient if no adsorption had taken place on the column.

front and that the shape and position of this front are not much altered by the presence of the adsorbed substance. Powerful desorbents are thus unsuited to the separation of members of such a group as the disaccharides. Here,
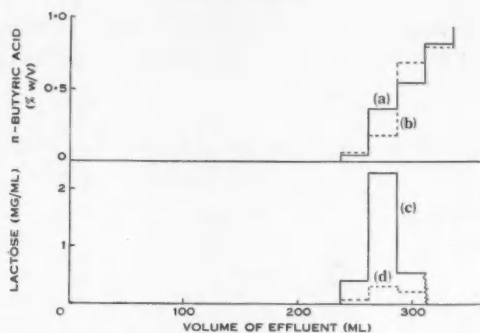
Fig. 5.—Relation between peak of desorbed substance and desorbent front for lactose and n-butyric acid using a small column at 28 °C.
Solution supplied to mixing chamber, 5 per cent. n-butyric acid. (a) Butyric acid front in the absence of lactose; (b) same front in the presence of 75 mg lactose; (c) distribution of 75 mg lactose; (d) distribution of 7·5 mg lactose ($\times 2$).
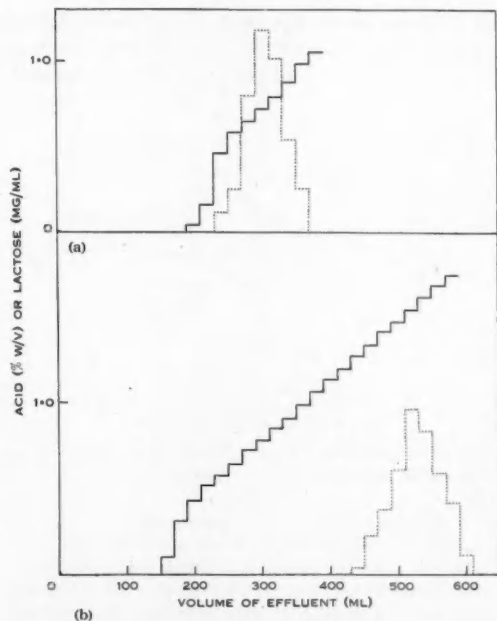
Fig. 6.—Desorption of 75 mg of lactose from the small column at 28 °C.
— Acid front. ··· Lactose peaks. Solution supplied to mixing chamber (a) 5 per cent. propionic acid; (b) 5 per cent. acetic acid.

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minimal fronting and the emergence of the substances to be separated as far away as possible from what front remains are required for useful results. Figure 6 shows how the lactose peak moves further back from the acid front as the series from n-butyric to acetic acid is descended ; as is evident from later figures formic acid is too poor a desorbent to give a sharp peak in a reasonable volume at the concentration levels studied.

(vii) *Effect of Concentration Gradient on Desorption.*—Two experiments were run to determine the effect of varying the concentration gradient of the desorbent under conditions otherwise fixed. One, the desorption of 75 mg of lactose from
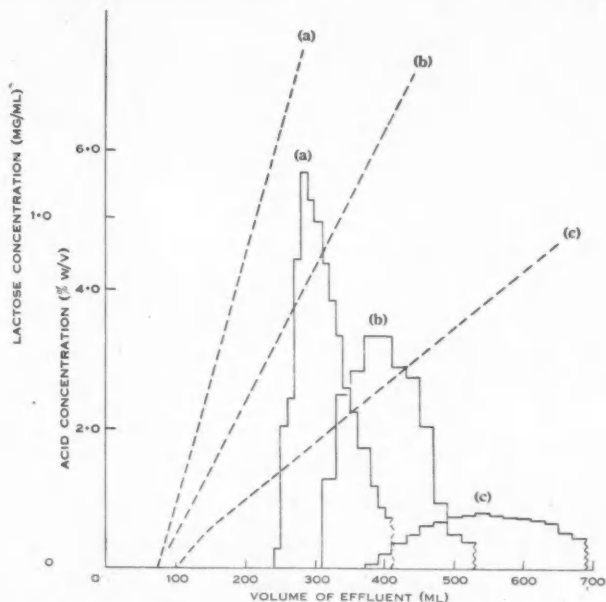
Fig. 7.—Desorption of 75 mg of lactose from the small column at 28 °C by varying concentration gradients of formic acid.

··· Acid gradient. — Lactose peaks. Solution supplied to the mixing chamber (*a*) 40 per cent. formic acid ; (*b*) 20 per cent. formic acid ; (*c*) 10 per cent. formic acid.

the small column by varying strengths of formic acid, is presented graphically in Figure 7. The conclusion from this experiment and one in which 500 mg of cellobiose was desorbed from the large column by varying gradients of ethanol is that the peak of desorbed substance appears at a volume of eluate where a certain constant weight of the desorbent has passed through the column (Table 6). Figure 7 shows that small experimental errors could make a large difference in the calculation of the amount of material which has passed at the crest of the peak and the agreement seems to be good enough to suppose that the hypothesis

E

M. A. JERMYN

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TABLE 6

EFFECT OF CONCENTRATION GRADIENT ON DESORPTION

</div>

Column	Hold-up Volume (ml)	Desorbed Substance (mg)	Desorbent	Concentration Supplied to Mixing Chamber (% w/v)	Volume of Eluate at Peak (ml)	Concentration of Eluate at Peak (% w/v)	Desorbent Passed at Peak (g)*
Large ..	450	Cellobiose 500	Ethanol	100	1520	8·5	45·5
				50	1960	5·5	41·5
				25	2650	4·0	44·0
				12·5	3920	3·0	45·0
Small ..	75	Lactose 75	Formic acid	40	290	7·72	8·3
				20	390	6·12	9·6
				10	540	3·88	8·9

* A small correction has been made in each case for the adsorption of the desorbing agent.

is true to the first approximation. It can readily be calculated that if the peak appears after V_x ml when an x per cent. solution of desorbent is supplied to the mixing chamber, V_0 being the hold-up volume of the apparatus then

$$V_y = V_0 + (V_x - V_0)\sqrt{(x/y)},$$

when a y per cent. solution is supplied (this calculation is of course restricted to conditions where the desorbent concentration at the peak $< x$ or y and the

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TABLE 7

RELATION BETWEEN PEAK CONCENTRATION OF 75 MG OF DESORBED LACTOSE AND WEIGHT OF PACKING IN THE COLUMN WITH A CONSTANT GRADIENT OF FORMIC ACID (20% w/v SUPPLIED TO THE MIXING CHAMBER)

</div>

Weight of Packing .	Volume of Eluate at Peak (ml)	Concentration of Formic Acid at Peak (w/v)	Desorbent Passed at Peak (g)	Desorbent/ Packing (g/g)
5	255	3·54	3·2	0·64
10	320	4·74	5·8	0·58
15	390	6·12	9·6	0·64
20	425	6·78	11·8	0·59
25	470	7·65	15·0	0·60

exponential gradient is very nearly a straight line). This method of prediction was later checked for a number of cases and found to give an approximately correct answer.

This result confirms the competitive nature of the replacement at the surface of the adsorbent. Table 7 shows that the amount of desorbent required to remove the same amount of desorbed substance from differing amounts of

charcoal is roughly proportional to the amount of charcoal. A similar square root formula to that derived above applies to variations in the amount of column packing.
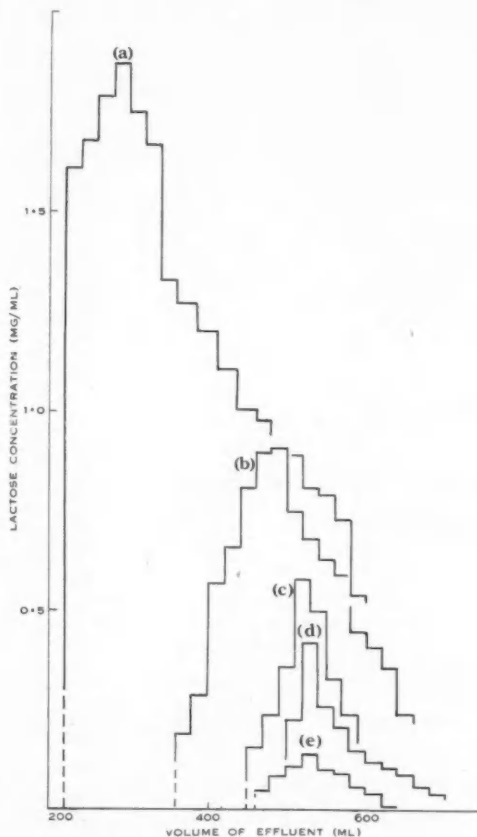
Fig. 8.—Desorption of various amounts of lactose from the small column at 28 °C.

Solution supplied to the mixing chamber 5 per cent. acetic acid. (a) 250 mg; (b) 125 mg; (c) 75 mg; (d) 40 mg; (e) 25 mg. The curves have been arbitrarily scaled up or down slightly to prevent overlapping.

(viii) *Effect of the Amount of Adsorbed Substance on Desorption.*—When the same concentration gradient of acetic acid was used to elute different amounts of lactose from the small column, the results shown in Figure 8 were obtained. That the results with the higher amounts of lactose were not produced by a large alteration of the shape of the gradient in the presence of the adsorbed

M. A. JERMYN

substance is shown in Figure 9. With amounts of lactose on the small column above 250 mg there was almost continuous leakage of lactose through the column with no sign of a definite peak. The smallest amounts that could be readily detected (about 2 mg) gave peaks at a position not sensibly different from 530 ml under the experimental conditions of Figure 8. It thus appears that there is a fixed position for a nearly symmetrical peak for amounts of adsorbed substance below a maximum (about 75 mg for lactose in this system), and skewing of the peak and shift of its position between this amount and the maximum that can
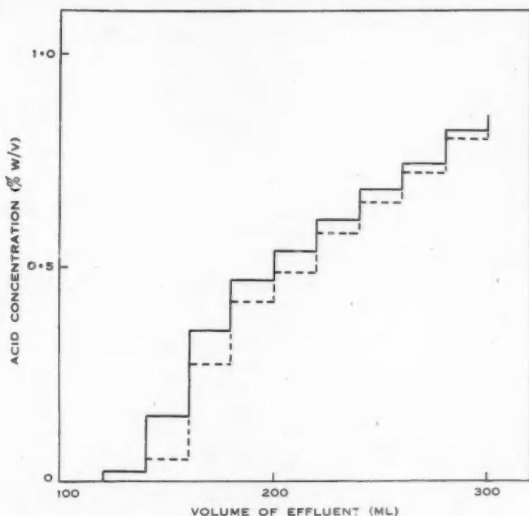
Fig. 9.—Concentration gradient formed when 5 per cent. acetic acid was supplied to the mixing chamber of the small column. ··· No adsorbed substance. — 250 mg of lactose adsorbed.

be held by the column. In this system, as in other chromatographic systems, there is thus a practical upper limit, considerably below the apparent hold-up value, to the amount of adsorbed substance which can be present on the column if consistent results are to be obtained. It may also be noted that there is always a considerable tendency for the desorbed sugar to "tail" as might be expected from the imperfect competition between acid and sugar shown in Table 3.

(ix) *Effect of Gradient Slope on Peak Height and Bandwidth.*—The effective separation of two substances by any chromatographic procedure depends not only on the distance between the peaks of their elution curves but also on the shapes of these curves. Where elution is due not to any chemical action but purely to competition between adsorbed substance and desorbent for the active surface, it could be expected that peak height will become lower as the gradient

of desorbent becomes flatter. Table 8 and Figure 7 show that in fact peak height is inversely proportional to distance travelled, that is, to the square root of the gradient. Further, since distance travelled and concentration of desorbent at the peak are inversely proportional, the peak height is proportional to the

TABLE 8

RELATION BETWEEN PEAK HEIGHT AND POSITION OF PEAK FOR ELUTION OF 500 MG OF CELLOBIOSE FROM THE LARGE COLUMN WITH ETHANOL GRADIENTS

Peak Height (relative)	$V_{peak}-V_0$ (ml)	Numerical Product of Columns (1×2)
590	1070	632
353	1560	535
242	2200	534
163	3470	565

concentration of the desorbent at the peak. This is what would be expected from the relationship

$$C_{Lac} = K \cdot \left(\frac{n_{Lac}}{n_{EtOH}} \right) \cdot C_{EtOH},$$

since n_{Lac} and n_{EtOH} are fixed by the conditions of the experiment. The relationship between peak height and peak position is therefore also explained by the mechanism of competitive adsorption.

TABLE 9

RELATION BETWEEN BANDWIDTH, PEAK HEIGHT, AND POSITION OF PEAK FOR THE ELUTION OF 500 MG OF CELLOBIOSE FROM THE LARGE COLUMN WITH ETHANOL GRADIENTS

1 Bandwidth (ml)	2 Peak Height (relative)	3 Numerical Product Columns (1×2)	4 $V_{peak}-V_0$ (ml)	5 Ratio 4 : 1
240	0·590	141	1070	4·5
450	0·353	160	1510	3·4
600	0·242	145	2200	3·3
800	0·163	131	3470	4·3

If it is assumed that the elution curves are in fact Gaussian curves of the same family then peak height and bandwidth (width of the elution curve at half peak height) should vary reciprocally, since the area under the curves (weight of sugar eluted) is constant. Although the elution curves depart considerably from this model (they are skew curves with relatively steep fronts and prolonged tails), it can none the less be seen that peak height and bandwidth are roughly inversely proportional and hence bandwidth and volume eluted at peak are directly proportional (Table 9).

It follows from these considerations that for a given pair of adsorbed substances at constant loading, a procedure that increases their separation will both decrease the height of the peaks and spread them out proportionately. In fact, if the separation is increased by a factor x, then the load on the column must be decreased by $1/x^2$ to give equally sharp peaks. Since the peaks will always appear after a certain weight of desorbent has passed through the column no alteration in the shape of the gradient can essentially alter this state of affairs. A similar conclusion has already been reached by Drake (1955).

A number of practical cases can be distinguished :

(1) *The Separation of a Number of Adsorbed Substances of Similar Affinity.* The best conditions will involve using the steepest gradient of the worst possible desorbent. The spread of the peaks as the separations are increased will have to be met by decreasing the load on the column (Figs. 12, 13).

(2) *The Separation of Adsorbed Substances of Widely Different Affinity.* A steep gradient is required of a desorbent which is just efficient enough to give sharp peaks with the most strongly adsorbed components of the mixture (Figs. 15 (a), 16).

(3) *The Separation of Adsorbed Substances falling into Groups with Similar Affinities.* This is the only case in which manipulation of the gradient to give a shape more complicated than the straight line or exponential curve is to be preferred since a flat gradient will give the same effect as a poor desorbing agent for one group and a steep gradient that of a good desorbing agent for another (Fig. 17).

(x) *Separation of Two Adsorbed Substances.*—It was concluded from the experiments reported above that the state of affairs required for good separations of adsorbed substances (sharp peaks well away from the solvent front) will be produced by using the steepest possible gradients of the worst possible desorbents. The only other factor that could upset this conclusion would be marked inter-action between the sugars, although this would not be expected if their adsorption is truly competitive (Table 4). Figure 10 shows the desorption of increasing amounts of sucrose and lactose under otherwise constant conditions ; the behaviour of lactose at (150/150 mg) is approximately that which would be expected for 150 mg of lactose alone except that the position of the peak is even further displaced from the " normal " position. At 75/75 mg the peak is near what would be expected for 150 mg of lactose from Figure 8.

The accuracy of the predictions made about the nature and concentration gradient of the desorbent can be checked by examination of the data recorded in Figure 11. Acetic acid, formic acid, and methanol form a series of desorbents of decreasing effectiveness and the results agree with what had been expected.

To illustrate the application of the results of this section, Figures 12 and 13 show the effect of small changes in technique on the separation of a mixture of seven disaccharides. By lengthening the column for a given amount of sugars and readjusting the gradient to the new column length (cf. also Figs. 11 (d), (f)) separations somewhat better than those of Figure 13 could certainly be obtained, but it seems that without such further devices as the addition of borate (Barker,

Bourne, and Theander 1955) the separations presented here are near the practical limit. Lactose and maltose do not appear to be separable by solvent gradients alone.

The data of Figures 12 and 13 may readily be used to check the validity of the procedures developed earlier for predicting the effect of changed conditions on the performance of the column. For the experiment illustrated in Figure 12, we have

$$\text{Volume of effluent at cellobiose peak} = 495 \text{ ml},$$
$$\text{Hold-up volume of column} = 75 \text{ ml},$$
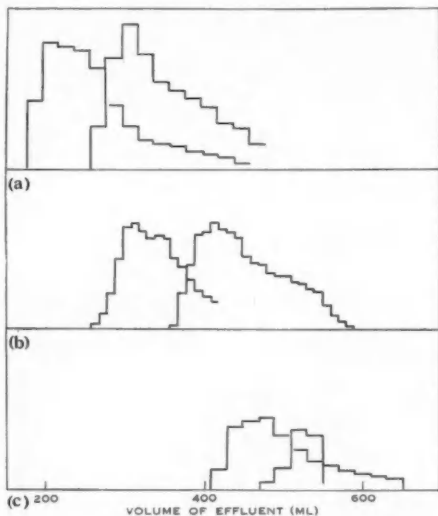
Fig. 10.—Separation of various amounts of sucrose (first peak) and lactose in the concentration gradient formed when 5 per cent. acetic acid was supplied to the mixing chamber.

(a) 150 mg/150 mg; (b) 75 mg/75 mg; (c) 25 mg/25 mg.

therefore, for the experiment of Figure 13 we have (treating the gradient as linear to the first approximation)

Volume of effluent at cellobiose peak =

$$75 + (495 - 75) \times \sqrt{(100/75)} \times \sqrt{(25/15)} \text{ ml} = 700 \text{ ml}.$$

Experimentally the peak occurs at 710 ml.

(b) Separation of Cellulose Oligosaccharides

(i) *Hydrolysis of Cellulose.*—A mixture of cellulose oligosaccharides was produced for these experiments by dissolving Whatman's " Ashless Cellulose Powder " in fuming hydrochloric acid (sp. gr. 1·21). After 10 min mixing at room temperature, the 10 per cent. (w/v) solution was held in a bath at 28 °C

M. A. JERMYN
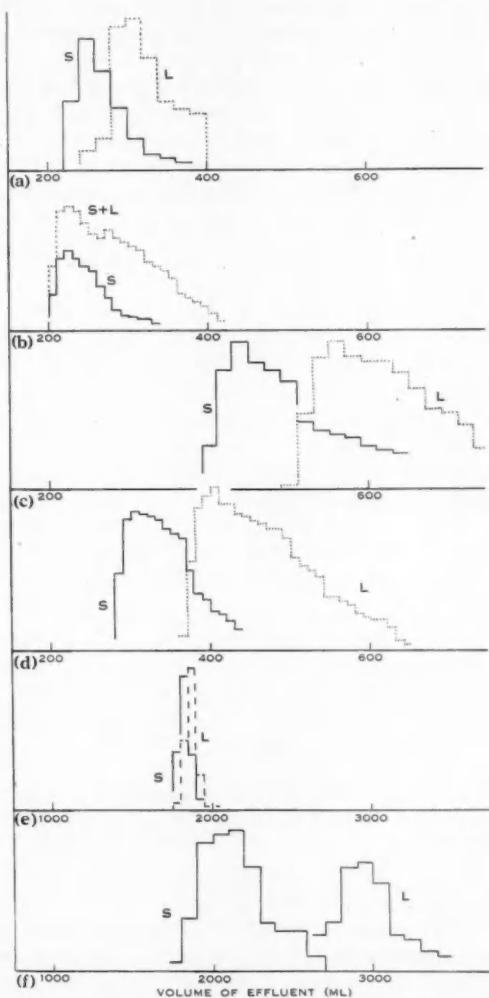
Fig. 11.—Desorption of mixtures of sucrose and lactose from charcoal columns.

(a) 75 mg/75 mg ; small column at 28 °C, 10 per cent. (w/v) acetic acid.
(b) 75 mg/75 mg ; small column at 28 °C, 40 per cent. (w/v) formic acid
(reducing sugar determinations impossible).   (c) 75 mg/75 mg ; small column
at 28 °C, 44 per cent. (w/v) methanol.   (d) 75 mg/75 mg ; small column at
28 °C, 88 per cent. (w/v) methanol.   (e) 500 mg/500 mg ; large column at
room temperature, 8 per cent. n-butanol.   (f) 500 mg/500 mg ; large column
at room temperature, 100 per cent. methanol (gives same gradient in pro-
portion to length as in (d)).

while hydrolysis proceeded.   Sampling showed that after 60 min at this temperature about 25 per cent. of the bonds were broken and application of the formula of Kuhn (1930) for the random hydrolysis of polymers showed that the first few oligomers should be present in approximately equal amounts (Table 10).   The
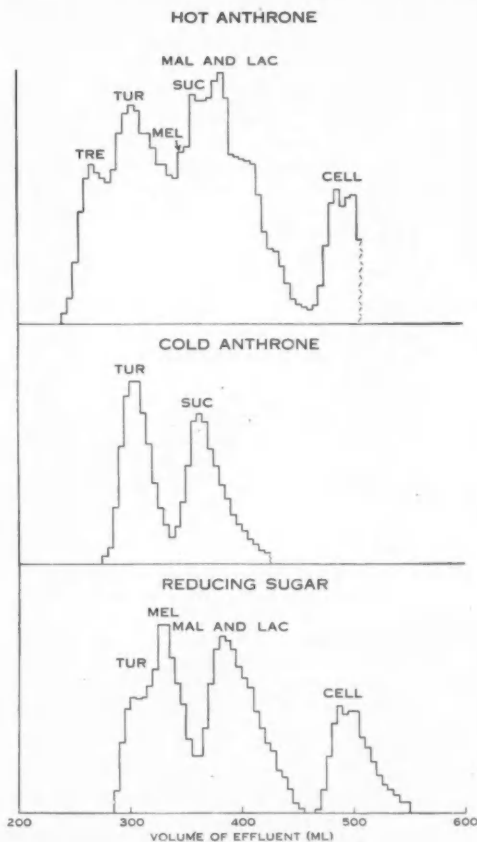
Fig. 12.—Separation of a mixture of 10 mg each of trehalose, turanose, melibiose, sucrose, maltose, lactose, and cellobiose on the small column at 28 °C.   Methanol (100 per cent.) was supplied to the mixing chamber.

hydrolysate was diluted with about 3 volumes of ice and water and applied directly to the head of the column.   The glucose and acid were washed through the column with water and passage of the eluting agent was not begun before the pH of the effluent fell to about 5.   The insoluble matter was ignored as it was gelatinous and difficult to filter and wash, and the presence of this material at
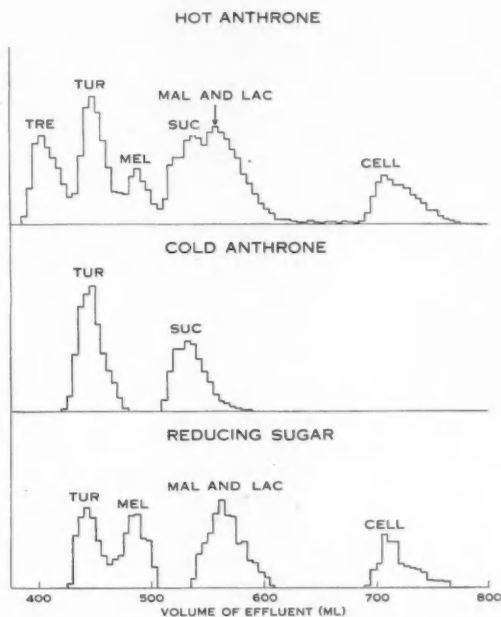
Fig. 13.—Separation of a mixture of 5 mg each of trehalose, turanose, melibiose, sucrose, maltose, lactose, and cellobiose on a " Darco G–60 " column at 28 °C. The small column packing had been increased to 25 g and the solution supplied to the mixing chamber reduced to 75 per cent. (v/v) methanol but the apparatus was otherwise the same as for Figure 12.

TABLE 10

DISTRIBUTION OF n-MERS AT 25 PER CENT. HYDROLYSIS OF CELLULOSE ACCORDING TO THE FORMULA OF KUHN (1930) COMPARED WITH ACTUAL DISTRIBUTION FOUND FOR FUMING HCl HYDROLYSIS

n	1	2	3	4	5	6	7	8
Percentage of mixture according to Kuhn's (1930) formula	6·2	9·4	10·5	10·5	10·0	8·9	7·8	6·7
Percentage of mixture as found by chromatography on charcoal (Fig. 15)	12·7	8·0	9·1	10·2	10·9	—	—	—

the head of the column did not seem to affect its working.   The amount of insoluble matter (about 22 per cent.) corresponds with insolubility in dilute HCl above $n=10$, using Kuhn's formula as a rough guide.
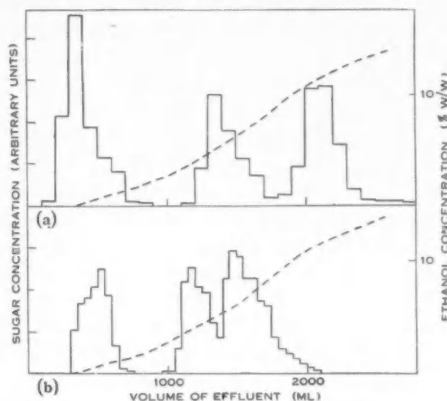
Fig. 14.—Separation of (*a*) glucose, cellobiose, and raffinose ; and (*b*) galactose, lactose, and melezitose on the large column in an ethanol gradient.
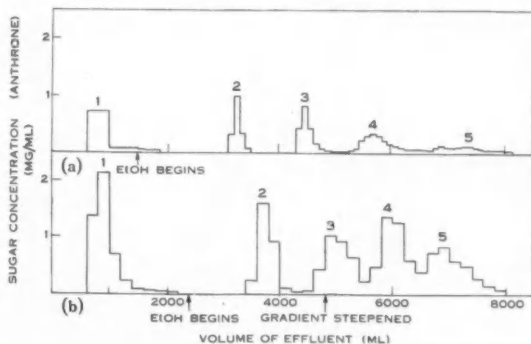
Fig. 15.—Separation of the components of a cellulose hydrolysate on the large column in an ethanol gradient.

(*a*) 42 per cent. HCl hydrolysate of 3 g of Whatman's " Ashless Cellulose Powder " ; and (*b*) the same from 9 g, with manipulation of the gradient.   The identity of the components was checked by comparing anthrone carbohydrate and reducing sugar values and is indicated by the number (=number of glucose residues in the molecule) against each peak.

This method of working has been preferred as simpler than others involving preliminary neutralization of the acid (Zechmeister and Toth 1931) or deacetylation of a partial acetolysate of cellulose (Whitaker 1954).
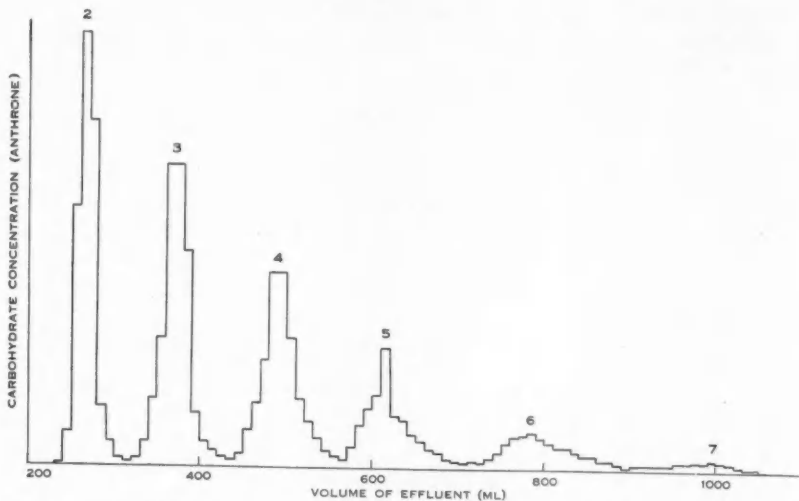
Fig. 16.—Separation of the components of a hydrolysate of 500 mg of cellulose on the small column at 28 °C.

Solution supplied to the mixing chamber water saturated with *n*-butanol. Acid and glucose had been removed from the column by water washing.
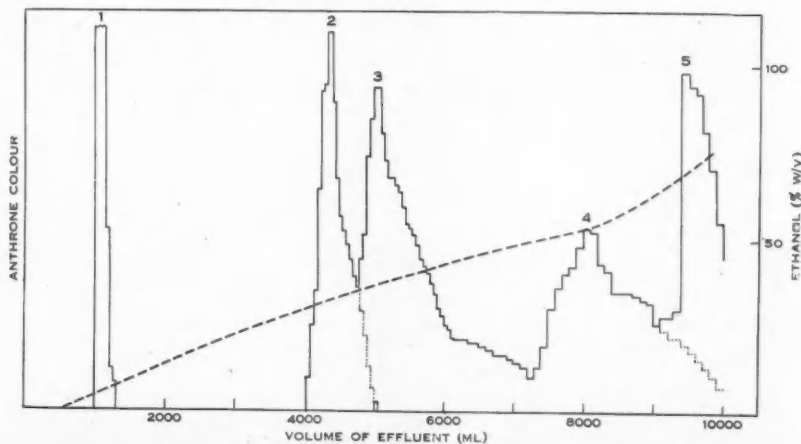
Fig. 17.—Separation in an ethanol gradient on the large column of 250 mg each of 1, β-glucoside ; 2, *p*-hydroxyphenyl β-glucoside (arbutin) ; 3, phenyl β-glucoside ; 4, *p*-nitrophenyl β-glucoside ; and 5, 1-naphthyl β-glucoside.

The – – – line represents the ethanol gradient. The · · · curves are derived from concurrent tests run to determine individual glucosides—Folin-Ciocalteau phenol determinations for arbutin and *p*-nitrophenol content from the nitrophenyl glucoside.

(ii) *Separation of Cello-oligoglucosides.*—Mixtures of mono-, di-, and tri-saccharides were readily separated in an ethanol gradient on the large column (Fig. 14).

When this technique was applied to a mixture of adsorbed cellulose oligo-saccharides it was found (Fig. 15 (*a*)) that the elution proceeded normally for the first three oligosaccharides but that cellotetraose gave a very skew peak and cellopentaose a long and indefinite hump. When the exponential gradient was altered to a sharp linear one as the cellotriose peak emerged from the column, cellotetraose and cellopentaose now appeared as sharp peaks, but there was no sign of cellohexaose and higher oligoglucosides.

Since the cello-oligoglucosides were thus much more strongly retained than the malto-oligoglucosides (Lindberg and Wickberg 1953), it was desirable to discover whether the oligomers above cellopentaose were actually irreversibly adsorbed on the column or merely so strongly held that a more powerful desorbing agent than ethanol was required. Figure 16 shows a run carried out on the small column using an *n*-butanol gradient. The hexaose and heptaose here behave almost exactly as the tetraose and pentaose did in the experiment of Figure 15 (*a*).

By freeze-drying suitable fractions from a large column operated with an *n*-butanol gradient it is possible to obtain pure cellotriose to cellohexaose in gram quantities. Beyond this it does not appear practical to go with techniques employing organic solvent gradients alone, since normal alcohols higher than *n*-butanol are limited by their low solubility in water while it is almost impossible to remove acids and bases without some hydrolysis or damage to the reducing group. A technique using a water-soluble alcohol as a carrier for a higher water-insoluble one might perhaps make the heptaose and octaose available.

(c) *Separation of Aryl Glucosides*

Like the oligoglycosides the aryl glucosides have a high affinity for charcoal and it should be possible to separate them readily in the same type of gradient. Figure 17 shows the separation of methyl β-glucoside and four aromatic β-gluco-sides by this method.

IV. Conclusions

It is not intended to attempt to formulate here a complete theory of the operation of gradient elution columns. However, it has been demonstrated that the desorption of sugars from charcoal columns by concentration gradients or organic substances dissolved in water can be explained by two experimentally determined facts :

(i) The sugar and the organic molecule are simple competitors for the same active surface.

(ii) For less than a certain maximum amount of adsorbed sugar, a fixed amount of desorbent will have passed when the peak concentration of desorbed sugar emerges from the column.

It is obvious that these observations will not hold in systems very different from those studied, and it seems rather that they represent one limiting case that will have to be included in any general theory of gradient elution columns.

V. Acknowledgment

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THE CARDIAC GLYCOSIDES OF *GOMPHOCARPUS FRUTICOSUS* R.BR.

II. GOMPHOSIDE

By T. R. WATSON* and S. E. WRIGHT*

Summary

Gomphoside is the second glycoside which has been isolated from *Gomphocarpus fruticosus* R.Br. grown in Australia. This compound analyses for the formula $C_{29}H_{44}O_8$ and contains neither methoxyl nor acetyl groups, but forms a diacetate, $C_{33}H_{48}O_{10}$, which is unstable to chromium trioxide in acetic acid. Although gomphoside gives a negative Keller-Kiliani reaction, a quantitative oxidation by periodic acid indicates the presence of two free adjacent hydroxyl groups in the molecule. Hydrolysis of gomphoside gives gomphogenin, $C_{23}H_{34}O_5$, which does not give a colour reaction with tetranitromethane. The acetylation of gomphogenin yields acetylgomphogenin, $C_{25}H_{36}O_6$, which is unstable to chromium trioxide in acetic acid.

Gomphoside and its derivatives have ultraviolet and infra-red absorption spectra typical of those of the normal digitaloid compounds. The reactions which have been carried out on these substances indicate the presence of an hydroxyl group in gomphogenin, besides the normal hydroxyl groups at C_3 and C_{14}, which is resistant to acetylation, but which can be oxidized by chromium trioxide in acetic acid. The nature of the carbohydrate is as yet unknown.

I. INTRODUCTION

The isolation of the cardiac glycoside, afroside, from *Gomphocarpus fruticosus* R.Br. has been described (Watson and Wright 1954, 1956). A second glycoside, which has been named gomphoside, was also obtained from this plant, but in much lower yield than afroside. Gomphoside and afroside were extracted from the plant material and separated by chromatography on neutral alumina (Watson and Wright 1956). The yield of gomphoside varied from approximately 2–10 per cent. of the total glycosides obtained from the various plant samples which were extracted.

Gomphoside (I), m.p. 234–242 °C, analysed for the formula $C_{29}H_{44}O_8$. (This compound was reported as having the formula $C_{35}H_{52}O_{10}$, but further analyses of gomphoside and acetylgomphoside have favoured the formula $C_{29}H_{44}O_8$ (Watson and Wright 1954).) It gave positive Raymond and Legal reactions and carbohydrate test, but a negative Keller-Kiliani reaction.

Gomphoside contains neither methoxyl nor acetyl groups. Acetylation with acetic anhydride in pyridine gave a diacetate (II), m.p. 252–255 °C, which analysed for the formula $C_{33}H_{48}O_{10}$, and which was unstable to chromic acid. Gomphogenin (III) was obtained from gomphoside by hydrolysis with 5 per cent. sulphuric acid in methanol-water (75 : 25). This compound analysed for the

* Department of Pharmacy, University of Sydney.

formula $C_{23}H_{34}O_5$, and gave positive Raymond and Legal reactions, but a negative tetranitromethane test.

The acetylation of gomphogenin gave a monoacetate (IV) which analysed for the formula $C_{25}H_{36}O_6$. Acetylgomphogenin (IV) was oxidized by chromium trioxide in acetic acid, but an insufficient quantity of the oxidation product was available for physical and elemental analyses. On the basis of these results the following partial structural formulae are proposed:

C$_6$H$_9$O(OR)$_2$

(I) R = H
(II) R = Ac

(III) R = H
(IV) R = Ac
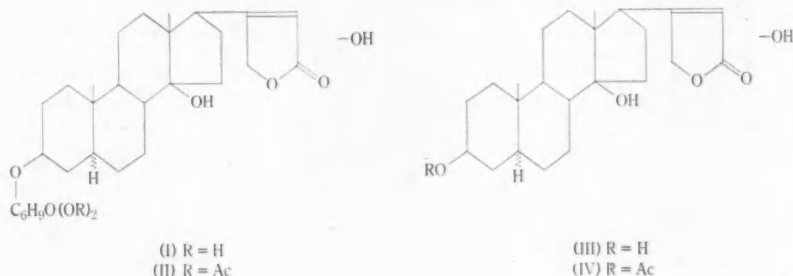
II. Discussion

As the ultraviolet absorption spectra of all the compounds mentioned have the typical absorption band of the butenolide ring of the digitalis-like glycosides (λ_{max}. 218–220 mμ, log ε approx. 4·2), the presence of this side-chain is assumed in each case. Further evidence for the presence of this unsaturated lactone ring is afforded by the positive reactions of these compounds to the Raymond and Legal reactions.

The ultraviolet absorption spectrum of gomphoside (I) (Fig. 1) differs from that of afroside, in the absence of any absorption in the 300 mμ region. This indicates the absence of a carbonyl (—CHO) group at C_{10}. Those digitalis-like cardiac glycosides which have a methyl group at C_{10} show no absorption in this region of the ultraviolet spectrum. Consequently, it is assumed that gomphoside (I) has a methyl group at C_{10} in the steroid nucleus. On acetylation, gomphoside forms a diacetate, acetylgomphoside (II), which indicates that there are two free hydroxyl groups in the molecule which may be esterified. However, this acetate was oxidized by chromic acid in acetic acid which indicates that there is an unacetylated primary or secondary hydroxyl group in the molecule.

As in the case of afroside, gomphoside (I) could not be hydrolysed by refluxing it in 0·1N sulphuric acid for 30 min. Also, when it was refluxed with Kiliani's mixture (acetic acid : water : hydrochloric acid, 3·5 : 5·5 : 1·0) the starting material was recovered unchanged. Gomphogenin (III) was obtained by refluxing gomphoside in 5 per cent. sulphuric acid in methanol-water (75 : 25) for 6 hr. This compound analysed for the formula $C_{23}H_{34}O_5$ which indicates that besides the four oxygen atoms normally accommodated in the lactone ring, the two hydroxyl groups at C_3 and C_{14}, there is an extra oxygen atom in the nucleus (cf. digitoxigenin $C_{23}H_{34}O_4$). On acetylation, gomphogenin forms the

monoacetate, acetylgomphogenin (IV), which must be due to the esterification of the C_3 hydroxyl group. Consequently the extra oxygen atom must be contained in a group which is not readily esterified. As the ultraviolet absorption spectrum of gomphogenin (III) shows no absorption apart from that of the butenolide ring at 218 mμ, it is reasonable to assume that the extra oxygen atom is not contained in an isolated chromophore. The most likely function of this oxygen atom is that of a sterically hindered hydroxyl group. The quantity of acetylgomphogenin available for chromic acid oxidation was too small to allow the isolation of the oxidation products. However, by carrying out the oxidation as a qualitative test, it was obvious that acetylgomphogenin was oxidized by the chromic acid. Thus it appears that gomphogenin contains an hydroxyl group which cannot be esterified but which can be oxidized. The
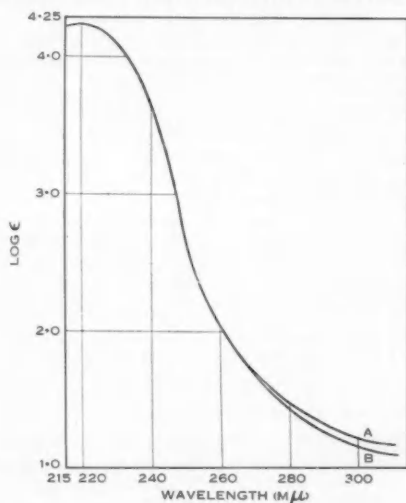
Fig. 1.—Ultraviolet absorption spectra in alcohol.
A, Gomphoside (I). B, Gomphogenin (III).

reactions which have been carried out do not indicate the position of this group, but by analogy with afroside (Watson and Wright 1956) it must be placed tentatively at C_{11} (β-axial).

The infra-red absorption spectra of gomphogenin, and acetylgomphogenin are shown in Figure 2. The absorption bands due to the C=O and C=C stretching frequencies of the butenolide ring of gomphogenin occur at 1745 cm^{-1} (5·73 μ) and 1633 cm^{-1} (6·12 μ) respectively. In acetylgomphogenin (IV) these bands occur at 1743 cm^{-1} (5·74 μ) and 1630 cm^{-1} (6·13 μ) respectively. Also, the absorption band of the C—O stretching frequency of the acetyl carbonyl group at 1250 cm^{-1} (8·00 μ) shows the complex structure associated with axially orientated substituents. (Owing to the low solubility of acetylgomphogenin in carbon disulphide and carbon tetrachloride, this spectrum was deter-

F

mined in chloroform solution.)   As all the known naturally occurring cardiac aglycones have a β-orientated hydroxyl group at C_3, for this group to be β and axial the A/B ring junction should be *cis*.   Thus in gomphogenin the A and B rings may have a *cis*-junction, which is the opposite configuration to that found for α-anhydroafrogenin (Watson and Wright 1956).

The partial formula assigned to the carbohydrate moiety of the glycoside requires it to be a dideoxyhexose.   However, as the Keller-Kiliani test was negative, the possibility of it being a 2,6-deoxyhexose appears to be unlikely. A quantitative oxidation of gomphoside by periodic acid indicated that 1 mole of the oxidizing agent was required for the reaction.   Therefore, the hydroxyl groups present in the carbohydrate must be on adjacent carbon atoms.   The constitution of the sugar is as yet unknown.
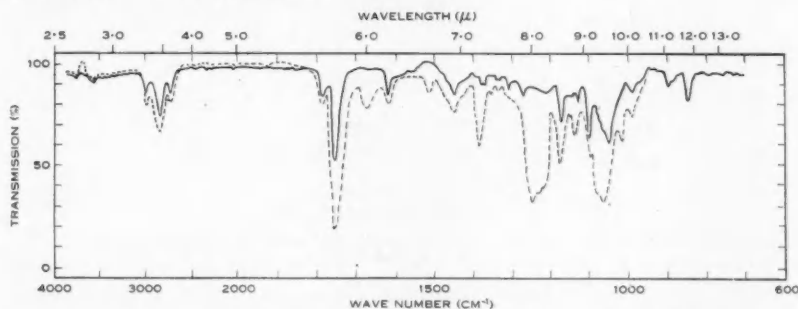
Fig. 2.—Infra-red absorption spectra. Saturated solutions in chloroform, NaCl prisms.
——— Gomphogenin. ···· Acetylgomphogenin.

III. Experimental

Melting points are uncorrected.   Ultraviolet absorption spectra were determined in ethanol on a Unicam S.P. 500 ultraviolet spectrophotometer, and infra-red spectra on a Perkin-Ehner double-beam instrument by Messrs. Timbrol Ltd., Sydney.   Optical rotation measurements were determined in a 1 dm micropolarimeter tube.   Analyses were carried out by Dr. K. W. Zimmerman, C.S.I.R.O. Microanalytical Laboratory.

The extraction and purification of gomphoside have been described by Watson and Wright (1956).

After three crystallizations from aqueous methanol, *gomphoside* was obtained as hexagonal plates, m.p. 234–242 °C, $[\alpha]_D$ +16·3±2° (c, 1·09 in MeOH) (Found : C, 66·7, 66·3, 66·2, 66·0 ; H, 8·2, 8·0, 8·4, 8·3%). Calc. for $C_{29}H_{44}O_8$: C, 66·9 ; H, 8·5%). Gomphoside gave positive Raymond and Legal reactions, but a negative Keller-Kiliani reaction. With sulphuric acid (80%), the following colour changes were noted (time in min) : 0 yellow, 30 orange, 60 orange-red, 120 lilac, 240 red-brown.   The total quantity of pure gomphoside obtained from the five extractions of plant material was 1·243 g.

Acetylation of Gomphoside.—Gomphoside (94 mg) was dissolved in dry pyridine (1 ml) and acetic anhydride (1 ml) added.   The solution stood at room temperature for 36 hr.   The acetylated material was extracted with chloroform.   The chloroform solution, after washing and drying, was evaporated to dryness under vacuum leaving 83 mg of colourless foam.

This foam was recrystallized three times from methanol-water, yielding 71 mg of colourless needles of *acetylgomphoside*, m.p. 252–255 °C, $[\alpha]_D^{20}$ +32±2° (c, 0·741 in $CHCl_3$) (Found : C, 65·2, 65·5 ; H, 7·7, 7·5 ; OAc, 14·2, 14·3%). Calc. for $C_{33}H_{48}O_{10}$: C, 65·6 ; H, 7·9, $2 \times$ OAc, 14·3%).

Chromium Trioxide Oxidation of Acetylgomphoside.—Acetylgomphoside (95·8 mg) was dissolved in acetic acid (2·0 ml) and a 2% solution of chromium trioxide (1·0 ml) in acetic acid was added. This solution stood at room temperature for 16 hr, after which another 0·25 ml of the chromium trioxide solution was added. (Reduction of the chromium trioxide had occurred as shown by the greenish colour of the solution.) The solution was allowed to stand until the reduction of the chromium trioxide was complete.

Sodium bicarbonate solution was added to the reaction mixture until the pH was 8–9, then extracted with chloroform. The chloroform solution was washed with water, then dried over anhydrous sodium sulphate. Evaporation of the chloroform under vacuum gave 22 mg of colourless foam. Recrystallization from methanol gave 15 mg colourless crystals, m.p. 250–255 °C, which were shown by mixed melting point and paper chromatography to be unchanged acetyl-gomphoside.

The aqueous solution remaining after the chloroform extraction was acidified to Congo red and reextracted with chloroform (5 × 10 ml). This chloroform extract was washed with water until the washings were neutral, dried over sodium sulphate, and the chloroform evaporated in a vacuum. Approximately 5 mg of oil remained which did not give a positive Raymond reaction. This residue was neutral but has not been identified.

Hydrolysis of Gomphoside.—Gomphoside (132 mg) was dissolved in 30 ml of Kiliani's mixture (water : acetic acid : hydrochloric acid, 5·5 : 3·5 : 1·0) and heated to 100 °C for 1½ hr. The solution assumed a pale yellow colour. On cooling, the solution was extracted with chloroform. The chloroform extract was washed and dried over anhydrous sodium sulphate. Evaporation to dryness in a vacuum gave 84 mg of yellow oil. Recrystallization of this oil from methanol-water gave 62 mg of white crystals, m.p. 230–242 °C, which were shown by mixed m.p. and carbo-hydrate test to be unchanged gomphoside. Gomphoside (107 mg) was dissolved in methanol (12 ml) and diluted with 10% sulphuric acid (12 ml) in 75% aqueous methanol. After refluxing this solution for 4 hr, the methanol was removed by vacuum distillation, and the hydrolysis product allowed to crystallize. A yield of 64 mg of colourless crystals was obtained, which on recrystallization from aqueous methanol gave 53 mg of colourless plates of *gomphogenin*, m.p. 266–270 °C, $[\alpha]_D^{23}$ +46·6±2° (*c*, 0·60 in EtOH) (Found : C, 70·0 ; H, 8·2%). Calc. for $C_{23}H_{34}O_5$: C, 70·7 ; H, 8·8%). This compound gave positive Raymond and Legal reactions, but a negative tetranitromethane reaction. With concentrated sulphuric acid, the following colour changes were noted (time in min) : 0 yellow, 30 orange, 60 orange-brown, 120 brown, 240 reddish violet.

Acetylation of Gomphogenin.—Gomphogenin (30 mg) was dissolved in pyridine (0·5 ml) and acetic anhydride (0·5 ml) was added. The solution was allowed to stand at room temperature for 48 hr, after which it was extracted with chloroform and treated in the manner described for the acetylation of gomphoside. The chloroform extract was dried and evaporated under vacuum. A yield of 26 mg of colourless foam was obtained, which on recrystallization from methanol gave 21 mg of colourless crystals of *acetylgomphogenin*, m.p. 150–156 °C, $[\alpha]_D$ +38±2° (*c*, 0·65 in CHCl$_3$) (Found : C, 69·2 ; H, 8·1 ; OAc, 8·8%. Calc. for $C_{25}H_{34}O_6$: C, 69·7 ; H, 8·0 ; 1-OAc, 10·0%).

Oxidation of Acetylgomphogenin by Chromium Trioxide in Acetic Acid.—Acetylgomphogenin (20 mg approx.) was dissolved in acetic acid (0·5 mg ; stable to chromium trioxide) and a 2% solution of chromium trioxide (0·5 ml) in acetic acid was added. A blank reaction, consisting of 0·5 mg of acetic acid to which was added the 20% chromium trioxide (0·5 ml) in acetic acid, was carried out at the same time. After standing at room temperature for 16 hr the solution containing the acetylgomphogenin had turned green, whereas the blank solution remained reddish brown. The 20% chromium trioxide (0·5 ml) in acetic acid solution was added to both blank and reaction mixtures, and these solutions were allowed to stand for a further 5 hr. After treatment of the reaction mixture in the manner described for the oxidation of acetylgomphoside, only a trace of a neutral material was obtained. This could not be identified.

Oxidation of Gomphoside with Periodic Acid.—Two portions of gomphoside were weighed into conical flasks, dissolved in methanol (10 ml), and 0·01523M periodic acid (10 ml) solution was added. One solution was allowed to stand at room temperature for 48 hr and the other for

72 hr. The solutions were diluted with water (10 ml) and neutralized with solid sodium bicarbonate. 0·100N sodium arsenite (20 ml) solution and 10% potassium iodide (1 ml) solution were added to each solution, the sodium arsenite in excess being estimated with 0·100N iodine solution.

(i) 41·27 mg = 0·0000793 g-mol (calc. for mol. wt. 520·64): Titrated after 48 hr. Volume of 0·100N I_2 required = 16·95 ml. By calculation, the quantity of periodic acid used in the oxidation = 0·0000725 g-mol.

(ii) 26·69 mg = 0·0000513 g-mol (calc. for mol. wt. 520·64): Titrated after 72 hr. Volume of 0·100N I_2 required = 18·05 ml. By calculation the quantity of periodic acid used in the oxidation = 0·0000550 g-mol.

IV. Acknowledgments

V. References

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SHORT COMMUNICATIONS

THE HALF–LIFE OF THORIUM C″*

By D. L. BAULCH,† H. A. DAVID,‡ and J. F. DUNCAN†

The range in gases of Th C″ atoms produced by α-recoil from Th B sources has recently been investigated by Baulch and Duncan (1957). Th C″ decays with a short half-life to stable ^{208}Pb. Each time measurements were made, a decay curve was determined, for extrapolation to zero time. In the course of this work, some 268 observations of the decay were made. Although the experiments were not originally designed for such a purpose, they provide a sufficient body of data for estimation of the half-life of Th C″ by statistical analysis.

Experimental

Sources of Th B were prepared by exposing a negatively charged platinum plate to finely powdered thorium hydroxide, which provided a source of thoron. Sources of Th B in radioactive equilibrium with its decay products, Th C, Th C′, and Th C″, which gave about 4000 counts/min with 5 per cent. counting efficiency, could thus be prepared. Each of these sources was placed parallel to and 2·90 mm from a silver collector, usually maintained at a positive potential of 300 V (Baulch and Duncan 1957). After standing in a vacuum of about 10^{-3} mm Hg pressure for 20 min, the collector was removed. The decay of the Th C″ collected was followed by taking readings of the integral numbers of counts obtained under an end-windowed Geiger counter at 1-min intervals, in favourable cases up to 20 min. Initial readings ranging from 100 to 1000 counts/min were obtained. The background, determined daily over a period of 10 min, was about 10 counts/min. Corrections for the dead time of the counter assembly were less than $\frac{1}{2}$ per cent.

Statistical Method

Estimation of the half-life was based on the decay occurring in the first 9 min only, this being the duration of several of the shortest series. Some of the 268 series of observations recorded showed features which could not be attributed to chance fluctuations in the counting rate. The following test was made of the validity of the results. Let n_{ti} denote the count obtained during the tth min ($t=1, 2, \ldots, 9$) of the ith experiment ($i=1, 2, 3, \ldots, 268$). Each series of nine points with coordinates ($t, y_{ti}=\ln n_{ti}$) was plotted, and lines with common

slope l were passed through the centroids $(5, \bar{y}_i)$. l is the average slope of the 268 least squares regression lines, and is the experimental estimate of λ, the disintegration constant. To control the deviations of points of a series from the regression line, parallel lines at a distance on the activity axis equal to $\pm 3 \times (8/9)^{\frac{1}{2}}s$ from the line through the centroid were drawn ; s^2 is the error mean square obtained by adding the 268 sums of squares of deviations from the individual regression lines and dividing by 268×7 (an estimate of the variance). Some 35 series which fell outside the areas bounded by these limits were rejected. The procedure was then repeated with new values of l and s. The new band was appreciably narrower, and another 22 series were rejected leaving 211 series. There was little further change in l and s in subsequent repetition of this procedure, which was therefore terminated.

The mean half-life, $t_{\frac{1}{2}}$ is $\ln 2/l$. The standard deviation in the half-life $\sigma_{t_{\frac{1}{2}}}$ was estimated from

$$\sigma_{t_{\frac{1}{2}}} = \frac{\sigma_l \times \ln 2}{(l)^2}$$

$$= \frac{s \times \ln 2}{(l)^2 [268 \sum_{t=1}^{9} (t - \bar{t})^2]^{\frac{1}{2}}} \quad (\bar{t} = 5).$$

These estimates are not affected by the grouping of counts chosen, nor is the fact that individual readings are about $0 \cdot 2$ per cent. larger than the disintegration rate which would be observed if it were possible to determine the disintegration rate instantaneously at $t = \frac{1}{2}, 1\frac{1}{2}, 2\frac{1}{2}, 3\frac{1}{2}, \ldots$, since this correction is constant for all readings (Cook and Duncan 1952).

Results and Discussion

The value of $3 \cdot 1$ min recommended by the International Radium Standard Commission (1931) is based on early work with considerable experimental error

TABLE 1
HALF-LIFE OF Th C''

Authority	Value (min)
Hahn and Meitner (1909) ..	$3 \cdot 1$
von Lerch and von Wartburg (1909)	$3 \cdot 0$
Albrecht (1919)	$3 \cdot 2$
International Radium Standard Commission (1931)	$3 \cdot 1$
Present work	
268 Series	$3 \cdot 090 \pm 0 \cdot 015*$
211 Series	$3 \cdot 099 \pm 0 \cdot 012*$

* Standard deviation.

(see Table 1). The fact that in the present work the two estimates reported, based on (i) all the 268 results and (ii) a selected 211 series, differ by only $0 \cdot 009$ min, gives one confidence in accepting the second place of decimals as

meaningful. The estimate from the 211* series is the more accurate. We therefore conclude that a safe estimate of the half-life is $3 \cdot 10$ min with a standard deviation of $0 \cdot 015$.

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THE DETERMINATION OF DEPOLARIZATION FACTORS WITH PHOTOELECTRIC RAMAN SPECTROMETERS*

By V. CRANMER† and R. L. WERNER†

The introduction of photoelectric methods has resulted in a greatly increased accuracy of measurement of the intensity of Raman lines and this in turn has facilitated the determination of depolarization factors (Rank 1947 ; Douglas and Rank 1948 ; Rank and Kagarise 1950 ; Stamm, Salzmann, and Mariner 1953).

The method usually employed is based on that of Edsall and Wilson (1938). Two polaroid cylinders, arranged so that the plane of polarized light is parallel to the axis of the cylinder in one and at right angles to this axis in the other, are placed in turn around the sample tube and the intensity of the Raman light being studied is then determined for each. The depolarization factor is given by

$$\rho_s = I_{\|}/I_{\perp},$$

where ρ_s is defined as : " The intensity ratio of the scattered light polarized along the direction of propagation of the exciting beam over the scattered light polarized perpendicular to this direction, when the exciting light is polarized at right angles to the tube length."

As has been shown by Douglas and Rank (1948) and Rank and Kagarise (1950), the values so measured are subject to several errors, one of the most serious being the angle of convergence of the radiation on the cell. The depolarization factor usually quoted however is ρ_n defined as : " The intensity ratio of the scattered light polarized along the direction of propagation of the exciting beam over the scattered light polarized perpendicular to this direction, when the exciting light is unpolarized."

The last-named authors have suggested that values of ρ_n can be obtained for any particular instrument by plotting experimentally determined values against known values of ρ_n, which have been determined by the method of

TABLE 1

SELECTED RAMAN LINES FOR THE CONSTRUCTION OF A CALIBRATION
CURVE FOR MEASUREMENT OF ρ_n

Compound	Raman Line (cm^{-1})	ρ_n
Carbon disulphide	654	0·25
Chloroform	366	0·13
	3020	0·32
Carbon tetrachloride	218	0·86
	313	0·86
	458	0·013
cycloHexane	802	0·063
Benzene	992	0·038
	606	0·86

parallel incident unpolarized light (no convergence of beam) and have been corrected for other errors which may occur. These authors further suggest that the correction curve should be a straight line.

Unfortunately, an examination of the available data for their suitability for use with photoelectric instruments has not previously been made. This has now been attempted by examining a large number of Raman lines for which ρ_n values have been reported. From these data a set has been selected (Table 1) which, it is believed, will enable a reliable estimate of ρ_n to be obtained from the measured values. The values given have been carefully chosen on the following basis :

(i) The Raman lines should be strong, so that a high signal-to-noise ratio can be maintained.

(ii) The lines should be reasonably symmetrical so that the measured intensity will not be sharply dependent on bandwidth.*

(iii) The lines should be as free as possible from nearby interfering lines which would prevent the base line being measured easily.

(iv) The compounds should be freely available in a pure form.

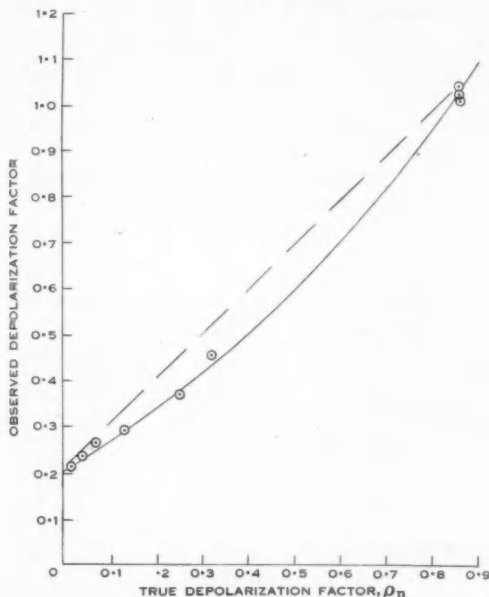(v) The reported ρ_n values must have been determined by a reliable method.

Fig. 1.—Observed depolarization factors obtained with a Hilger recording Raman spectrometer, plotted against true depolarization factors ρ_n.

Note.—If the experimental method were free from error the observed results would be a set of ρ_z values.

In Figure 1 experimental values measured with a Hilger recording Raman spectrometer (6 cm^{-1} slit-width) are plotted against the values of ρ_n recommended. It will be seen that a smooth curve is obtained. The dotted line indicates the position of the sine-cosine relation of Rank and Kagarise (1950). It is not surprising, considering the approximate nature of their derivation, that some departure from a linear relationship occurs.

* The authors' attention has been drawn to Marrinan and Sheppard's (1954) method, namely, that measurement of the area under the curve rather than peak height would obviate the effect of slit-width. However, in view of Douglas and Rank's (1948) observation that the polarization varies across the width of the line, we prefer to use peak height.

It is suggested that until such time as the sensitivity of photoelectric instruments is increased to a point where polarization measurements become practicable by the use of parallel incident polarized light, the values given will enable reasonable estimates of ρ_n to be made.

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THE PREPARATION OF SOME TRIALKYL ESTERS OF ORTHOBORIC ACID*

By K. G. O'BRIEN†

The tri-n-butyl-, tri-sec.-butyl-, and tri$cyclo$hexyl borate esters were prepared by the azeotropic method of Anderson, O'Brien, and Reuter (1952). All attempts to prepare tri-$tert$.-butyl borate in this way were unsuccessful, invariably producing a compound which appeared to be related to the trimethoxyboroxol described by Goubeau and Keller (1951). An account of the preparation and properties of trialkoxy boroxols is to be published.

The esters mentioned above have been used to study the infra-red spectra of borate esters (Werner and O'Brien 1955).

TABLE 1

PHYSICAL PROPERTIES AND ANALYTICAL VALUES OF SOME ALKYL BORATE ESTERS

Compound	Boiling Point (°C/mm)	$n_D^{22 \cdot 5}$	Yield (%)	Molecular Composition	Mol. Wt.
Tri - n - butyl b o r a t e, $C_{12}H_{27}O_3B$	136/30	1·4806	80	Calc.: C, 62·6; H, 11·8; B, 4·7% Found: C, 62·6; H, 11·8; B, 4·8%	Calc. 230 Found 220
Tri - sec. - butyl b o r a t e, $C_{12}H_{27}O_3B$	92/10	1·3950	75	Calc.: C, 62·6; H, 11·8; B, 4·8% Found: C, 62·6; H, 11·9; B, 4·8%	Calc. 230 Found 236
Tri - $cyclo$hexyl b o r a t e, $C_{18}H_{33}O_3B$	323–326/750	Solid, m.p. 56·4 °C	88	Calc.: C, 70·1; H, 10·3; B, 3·6% Found: C, 69·8; H, 10·5; B, 3·6%	Calc. 307 Found 306

Experimental

(i) *Azeotropic Distillation.*—The alcohol (0·6 mol), boric acid (0·2 mol), and benzene (300 ml) were heated in the apparatus described by Anderson, O'Brien, and Reuter (1952) until there was no further apparent evolution of water.

The solvent and alcohol in excess were distilled off at atmospheric pressure followed by the ester. Tri-n-butyl-, tri-sec.-butyl-, and tri$cyclo$hexyl borate esters were prepared by this method and analysed to give the results shown in Table 1.

* Manuscript received October 8, 1956.

† School of Applied Chemistry, N.S.W. University of Technology, Broken Hill.

(ii) *Azeotropic Fractional Distillation.*—The fractionation still consisted of a pot, a column (length 2 ft, internal diameter $\frac{1}{2}$ in.) packed with $\frac{1}{8}$ stainless steel Dixon rings (Dixon 1949), and a weir type reflux ratio head (McIntyre 1933). This head enabled the water to be readily separated from the azeotropic mixture.

TABLE 2

PHYSICAL PROPERTIES AND ANALYTICAL VALUES OF SOME ALKYL BORATE ESTERS

Compound	Boiling Point (°C/mm)	$n_D^{22.5}$	Yield (%)	Molecular Composition	Mol. Wt.
Trimethyl borate, $C_3H_9O_3B$	69/750	1·3585	55	Calc.: C, 34·7; H, 8·7; B, 10·6% Found: C, 35·0; H, 8·8; B, 10·8%	Calc. 104 Found 100
Tri - n - propyl borate, $C_9H_{21}O_3B$	180–183/760	1·4266	45	Calc.: C, 57·5; H, 11·2; B, 5·8% Found: C, 57·3; H, 11·0; B, 5·9%	Calc. 188 Found 180
Tri - tert. - butyl borate, $C_{12}H_{27}O_3B$	65–66/11	1·3869	44	Calc.: C, 62·6; H, 11·8; B, 4·7% Found: C, 62·6; H, 11·9; B, 4·8%	Calc. 230 Found 234
Tri - n - amyl borate, $C_{15}H_{33}O_3B$	154/16	1·4205	80	Calc.: C, 66·2; H, 12·1; B, 4·0% Found: C, 64·6; H, 11·5; B, 3·9%	Calc. 271 Found 260
Tri - n - heptyl borate, $C_{21}H_{45}O_3B$	192/4–6	1·4355	88	Calc.: C, 70·8; H, 12·6; B, 3·2% Found: C, 70·8; H, 12·6; B, 3·3%	Calc. 355 Found 348
Tri - (2 - ethyl)- hexyl borate, $C_{24}H_{51}O_3B$	348–352/745	1·4365	73	Calc.: C, 72·4; H, 12·8; B, 2·8% Found: C, 72·1; H, 12·7; B, 2·8%	Calc. 397 Found 405
Tri - 2 - octyl borate, $C_{24}H_{51}O_3B$	170/1	1·4275	83	Calc.: C, 72·4; H, 12·9; B, 2·8% Found: C, 72·1; H, 12·9; B, 2·7%	Calc. 397 Found 400

The alcohol (0·8 mol) and boric acid (0·2 mol) were heated until the boil up rate was just below the flood point of the column. The reflux ratio was then set to 20 : 1 and the distillation continued until the temperature at the still head rose to that of the alcohol being esterified. The bulk of the excess alcohol was then distilled off and the residue distilled *in vacuo* or at atmospheric pressure, depending upon the alcohol. The yield, boiling point, refractive indices, and analytical values of the resulting ester are presented in Table 2.

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Reynol...
lite IR...
column...
with 0...
graphy...
(50 : 50...
indicat...
glutari...
acid.

Th...
(3 g) cr...
C, 48·...
Shikim...
materi...
(" Nuje...
$[\alpha]_D^{20}$ —...

* M...
† D...
‡ D...
of Chem...

In the case of the trimethyl borate, the ester and methanol formed a minimum boiling point azeotrope which was collected at 54–60 °C. The azeotrope was treated with ice-cold sulphuric acid as recommended by Webster and Dennis (1933) and then fractionated to give the pure ester, b.p. 69 °C/745 mm.

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THE ISOLATION OF SHIKIMIC ACID FROM *EUCALYPTUS CITRIODORA* HOOK.*

By E. F. L. J. ANET,† A. J. BIRCH,‡ and R. A. MASSY-WESTROPP‡

Young leaves of *Eucalyptus citriodora* Hook. (10 kg) were frozen in liquid air immediately on picking, were pulped, and immediately extracted with ethanol (95 per cent.) to give an approx. 50 per cent. ethanolic solution, which was passed through a cation-exchange resin " Zeocarb 225 " to remove bases and amino acids. The method then employed was essentially that of Anet and Reynolds (1955). The acids were collected on an anion-exchange resin " Amberlite IR–4B " and displaced with $0 \cdot 1$N hydrochloric acid onto a series of four columns of " Amberlite IRA–400 ". The acids on the columns were displaced with $0 \cdot 1$N hydrochloric acid and 20 ml fractions collected. Paper chromatography using methyl ethyl ketone : cineole : formic acid (85 per cent.) : water (50 : 50 : 20 : 16 v/v) together with the sequence of emergence from the columns indicated the presence of the following acids in order of elution : R_1, quinic acid, glutaric acid, succinic acid, malic acid, citric acid, phosphoric acid, hydrochloric acid. Small amounts of unknown acids were also present.

The fractions containing the acid R_1 were evaporated and the product (3 g) crystallized from water, m.p. 183 °C, $[\alpha]_D^{25}$ −178° (*c*, $0 \cdot 04$ in water) (Found : C, $48 \cdot 1$; H, $5 \cdot 8\%$. Calc. for $C_7H_{10}O_5$: C, $48 \cdot 3$; H, $5 \cdot 8\%$) equiv. wt., 175. Shikimic acid from *E. citriodora*, m.p. 183–186 °C was undepressed by authentic material, m.p. 183–187 °C. In addition the infra-red spectra of the two specimens (" Nujol " mull) were identical. Authentic shikimic acid has m.p. 184 °C, $[\alpha]_D^{20}$ −176° (*c*, $0 \cdot 02$ in ethanol), equiv. wt., 174 (Grewe and Lorenzen 1953).

The presence of comparatively large quantities of shikimic acid and quinic acid in rapidly developing leaves is of interest in connection with the probable incorporation of these substances into phenylalanine, tryptophan, and lignin (e.g. Davis 1953).

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